EFFECT OF GINSENG SAPONIN ON PITUITARY-ADRENAL AXIS

BY

LAU KWAI-LING

B.SC. (NATIONAL TAIWAN UNIVERSITY)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN
BIOCHEMISTRY

MAY, 1980

DEPARTMENT OF BIOCHEMISTRY
THE CHINESE UNIVERSITY OF HONG KONG

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Dr. Y. C. Kong for his valuable guidance throughout my graduate study, and much helpful criticism and advice as regards the original text.

My grateful thanks are due to Professor U. Sankawa for his valuable discussions and for a generous gift of purified ginsenoside R_{g1} . I would also like to express my appreciation to Dr. H. Saito for his suggestions and discussions. I am further indebted to Dr. T. Akiyama for his instructions in phytochemical study of ginseng.

My sincere thanks are due to Dr. C. Y. Lee and Y. M. Choy for their independent reading of this manuscript, and to Dr. P. But for his informative suggestions.

I gratefully acknowledge the helpful assistance and fruitful discussions of Mr. T. T. Yip throughout the entire period of this investigation.

It is also my pleasure to extend my gratitude to extend my gratitude to Messrs. C.L. King, K. H. Ng, Miss Y. P. Law, and Miss N. B. Lau for their skilful assistance and profitable discussions.

ABSTRACT

Ginseng is a traditional herbal medicine which is highly appreciated by the people of China, Korea , and Japan. It has been found to possess many pharmacological effects. Of particular interests in modern day context is its anti-stress effect.

In the present study, total ginseng saponin fraction was administered to rats intraperitoneally in chronic doses and chronic-acute doses. It has been able to confirm that the total ginseng saponin fraction increased serum corticosterone level by 1.6 fold. However, this change seemed to be significant only when chronic-acute doses of ginseng saponin were administered over 3 days. It was further observed that ginseng saponin could change the distribution of corticosterone in the brain after chronic-acute treatment. There was a decrease in pituitary corticosterone level by 1.8 fold, and an increase in cerebrum corticosterone level by 1.7 fold. The results are statistically significant. Hypothalamus corticosterone level was not affected. However, the above parameters remained unchanged in the chronic group after ginseng treatment, indicating the acute effect of ginseng saponin on the rats.

Nevertheless, in the treated animals including the chronic group and chronic-acute group, total ginseng saponin could augment the response of adrenal gland in vitro

to different doses of ACTH over 100-fold range. Also, the binding capacity of liver binding protein was enhanced in both treated groups. The binding affinity was the same between control and treated animals. From these results, the chronic effect of ginseng saponin is demonstrated.

Binding capacity and affinity of serum CBG (corticosteroid-binding globulin) was also studied, but no difference was observed between control and treated animals in both groups.

These findings suggest that ginseng saponin can sensitize the pituitary-adrenal axis as a contribution to prepare the rat against stress.

TABLE OF CONTENTS

1. INTRODUCTION

Life consists of a series of micro and macro adaptations to a constantly changing internal and external environment. Life is a continuous confrontation with different problems in a condition that can be broadly described as stress. The body is consistently withstanding various kinds of chemical, physical and emotional stressors, such as shock, restraint, isolation, agression, cold, heat, hunger, tissue damage, etc. When the individual is subjected to chronic stress or recurrent stress, a number of diverse noxious effects may begin to manifest themselves and the body function may be altered reversibly or irreversibly.

Ginseng is well known among the Chinese people and other culturally related ethnic groups in East Asian countries since ancient time. It has been used traditionally for its anti-stress action. However, the scientific rationale of this ginseng action remained unknown although many studies and observations suggested the possibility that ginseng might simply reduce mortality through its influence upon stress mechanism. It is not until the middle of the 20th century that ginseng and glucocorticoid were first correlated.

The present study attempts to explore the action of ginseng on the pituitary-adrenal axis. The present data are not intended to afford a conclusion of the ginseng effects through glucocorticoid action, they are rather

intended to document a distribution pattern of corticosterone in the rat after ginseng treatment. This constitutes a preliminary step to gain more insight in the action mechanism of ginseng, with special reference to its anti-stress property.

In the present study, attention has been focused on the corticosterone level in serum and pituitary as well as other parts of the brain. Studies are extended to the corticosterone binding proteins in liver and serum. As a further effort to evaluate the ginseng effect on the adrenocortical system, steroidogenesis in the adrenals is also investigated.

1.1. GINSENG IN CHINESE MEDICINE:

Ginseng is one of the medicinal plant products discovered by the Chinese people since prehistoric times. In their struggle with hunger and diseases, the early people in North China found that the fleshy roots in the forest shades could give strength and vigor. While this special property of ginseng is passed on by word of mouth, certain myths were developed mainly influenced by the doctrine of signature. It was therefore suggested that ginseng root is a miniscule human form absorbing the essence from the earth, hence its tonic property. The first written account of ginseng appeared in the Shen Nung Pents'ao Ching (神農本草經), the first Chinese pharmacopoeia accredited

to the Divine Plowman (Shen-Nung 神農). The authorship of this pharmacopoeia remained obscure, it was probably first published in late Han dynasty (2-300 A.D.). In this work, the description of ginseng is short and concise. It consists of 44 words which give the name ginseng with two synonyms, the taste and property, the habitat, and medicinal function of the drug:

"Ginseng is also called Jen-hsien (人衘 Man Gag), or Kuei-kai (鬼蓋 Demon's Umbrella). It tastes sweetish, and its property is slightly cooling. It grows in the gorges of the mountains. It is used for repairing the five viscera, quietening the spirit, curbing the emotion, stopping agitation, removing noxious influence, brightening the eyes, enlightening the mind, and increasing the wisdom. Continuous use leads one to longevity with light weight." (Hu, 1977).

The term ginseng stands for two Chinese ideograms (人參). The character Jen (gin, 人) stands for man, and Shen (seng, 參) stands for essence. This refers to the more or less human form of the root and its tonic property.

In "Shang Han-Lun" by Chang Chung-Ching (150-219 A.D.), the first practical guide to clinical Chinese medicine, the use of ginseng was incorporated in several prescriptions. The knowledge in ginseng accumulated with the years. In the books published in Liang Dynasty (梁 502-555

A.D.), such as Pênts'ao Chi Chu (本草集注) and Ming I Pieh Lu (名醫別錄), the centers of production, the time for gathering the roots, and the morphological characters of the plants were given. By T'ang Dynasty (唐 618-905 A.D.), ginseng was an article of tribute presented to the royalty. In Sung Dynasty (宋 960-1126 A.D.), the use of ginseng became privilege of the rich. As time went on the demand for ginseng gradually increased while the production decreased. In Ming dynasty, Pênts'ao Kang Mu (本草綱目) by Li Shi Chen was published (1596), the properties and clinical functions of ginseng were fully described.

1.2. BOTANICAL CHARACTERISTICS OF GINSENG: (Hu, 1977)

The ginseng plant belongs to the Araliaceae family. The commercial product of ginseng comes from two separate species; they are Panax ginseng C.A. Meyer, which is the source of the Chinese, Korean and Japanese ginseng; and Panax quinquefolius L., which is the source of American ginseng.

Morphologically, ginseng is a perennial herb with short underground stem (rhizome) associated with a fleshy white root, a single annual stem bearing a whorl of palmately compound leaves, and a terminal simple umbel of small 5-merous flowers. The flowers are soon followed by pea-sized fruits developed from inferior ovaries. The fruits are red when ripe.

Ginseng plants begin to flower at the age of three or four. One common variation that occurs in ginseng plants is that the plants sometimes produce perfect flowers, and sometimes produce separate staminate and pistillate flowers instead. A plant that has perfect flowers will produce seed. The ginseng plant reproduces itself in nature by seed only. Another rarity in the ginseng plant that is occasionally encountered is the presence of a double embryo seed. One double embryo seed will give rise to two separate plants.

The ginseng roots are corpulent. They are composed of a tap-root with two to five lateral roots. The roots are light yellowish white in color. The plants are vigorous in producing lateral roots. The growth and the shape of the root also vary according to the age of the plants.

1.3. CHEMICAL STUDIES ON CONSTITUENTS OF GINSENG:

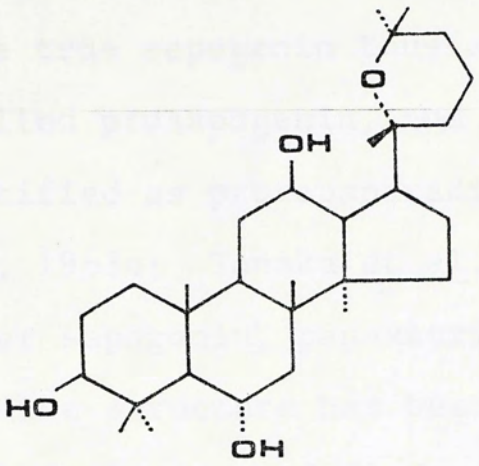
1.3.1. SAPONINS:

The dried roots and rhizomes of ginseng contain many physiologically active constituents. Among these, saponin is the major component which has been extensively studied.

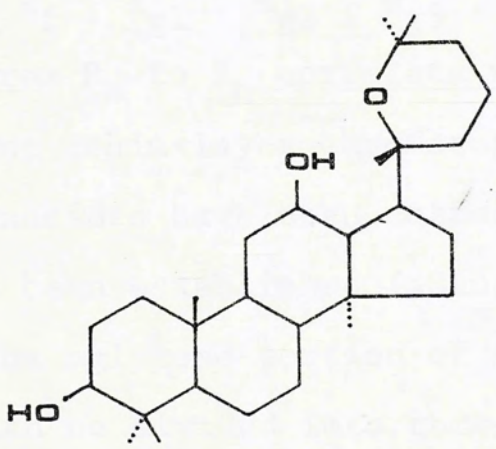
A number of ginseng saponin glycosides have been isolated from methanol extracts of ginseng roots and identified by Soviet workers (Elyakov et al, 1964; 1965; 1968). These saponins are called panaxosides. In 1964, Elyakov reported the successful isolation of saponin glycosides,

panaxosides A, B, C, D, E, and F. According to their structures, these six ginseng saponin glycosides are divided into two groups. Panaxosides A, B, and C belong to one group and yield a common non-sugar substance (aglycone) called panaxatriol (Structure I) after acid hydrolysis, while panaxosides D, E, and F belong to another group that yields panaxadiol (Structure II) after acid hydrolysis. None of the six panaxosides contains identical sugars. Panaxoside A consists of three molecules of glucose ; panaxoside B, two glucoses and one rhamnose; panaxoside C, three glucoses and one rhamnose; panaxoside D, four glucoses; panaxoside E, four glucoses and one arabinose; and panaxoside F, six glucoses (Elyakov et al, 1964).

Professors Fujita, Itokawa, and Shibata of University of Tokyo are the most eminent ginseng researchers. In 1962, they were able to isolate two types of new compounds from the methanol extracts of ginseng: sapogenin (aglycone) and pro-sapogenin (Fujita et al, 1962). Sapogenins are normally obtained when the saponin mixtures are treated with hot hydrochloric acid in methanol. It was later confirmed that the sapogenins thus obtained were not genuine sapogenins (aglycones), but actually an acid hydrolysed products of ginseng saponins. The structure of sapogenins was identified as panaxadiol (Structure II) (Fujita et al, 1962; Shibata, Fujita and Itokawa, 1962; Shibata et al, 1963a; 1963b). The genuine sapogenin of ginseng was even-



Structure I Panaxatriol

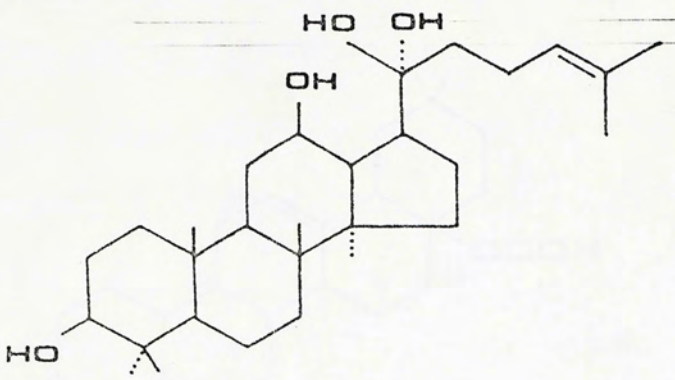


Structure II Panaxadiol

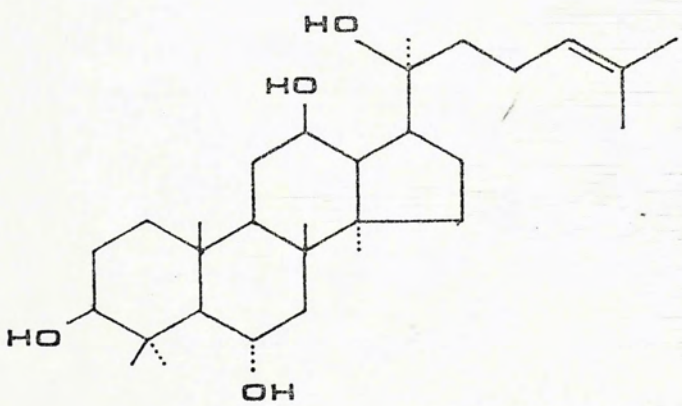
tually obtained when the mixture of ginseng saponins was hydrolysed under mild conditions (0.7% sulfuric acid in methanol). The true sapogenin thus obtained by the Japanese workers was called prosapogenin, and the chemical structure of it was identified as protopanaxadiol (Structure III) (Shibata et al, 1963c; Tanaka et al, 1964).

Another sapogenin, panaxatriol (Structure I) was also obtained, the structure has been confirmed, similarly, to be an acid hydrolyzed product during isolation. The genuine aglycone would be protopanaxatriol (Structure IV) (Shibata et al, 1965).

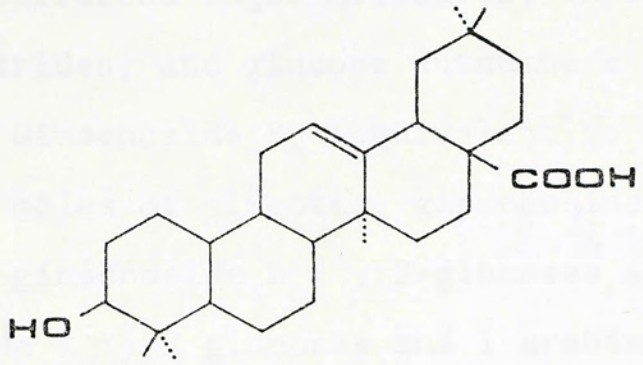
In 1974, Shibata and his collaborators (Sanada et al, 1974a; 1974b) successfully isolated 13 ginsenosides from ginseng root extract. They are: R_o , R_a , R_{b1} , R_{b2} , R_c , R_d , R_e , R_f , R_{g1} , R_{g2} , R_{g3} , R_{h1} , R_{h2} . The ginsenosides from R_o to R_h correlate with the increasing R_f-values of the thin-layer chromatography. Nine of the total 13 ginsenosides have been characterized, and their stuctures have been established (Sanada et al, 1974a, 1974b). Depending on the aglycone portion of the molcule, these ginsenosides can be divided into three groups. Ginsenoside R_o gives oleanolic acid (aglycone), while ginsenosides R_a , R_b , R_c , R_d give panaxadiol and ginsenosides R_e , R_f, R_g and R_h give panaxatriol after acid hydrolysis. Oleanolic acid (Structure V) is a pentacyclic, oleanane-type triterpene compound . Ginsenosides of the R_b and R_g



Structure III Protopanaxadiol



Structure IV Protopanaxatriol



Structure V Oleanolic acid

groups, on the other hand, are tetracyclic dammarane-type triterpenes.

Besides sapogenins each of these ginsenosides contains different sugar molecules, though they all have monosaccharides, and glucose outnumbers arabinose and rhamnose. Ginsenoside R_a (equivalent to Panaxoside F) carries 6 moles of glucoses; ginsenoside R_{b1} carries 4 glucoses; ginsenoside R_{b2} , 3 glucoses and 1 arabinose; ginsenoside R_c , 3 glucoses and 1 arabinose; ginsenoside R_d , 3 glucoses; ginsenoside R_e (equivalent to panaxoside B), 2 glucoses, and 1 rhamnose; ginsenoside R_f , 2 glucoses; ginsenoside R_{g1} , 2 glucoses; ginsenoside R_{g2} , 1 glucose and 1 rhamnose; ginsenoside R_o , 2 glucoses and glucuronic acid (Sanada et al, 1974a ; 1974b). Later, R_{b3} was also determined to have 3 glucoses and 1 xylose (Sanada and Shoji, 1978) , R_{h1} , 1 glucose (Yahara et al, 1979).

1.3.2. GINSENG OIL AND PHYTOSTEROL:

Ginseng oil contains volatile oil——panacene and β-elemene, and non-volatile oil——panxynol. Phytosterols extracted from Chinese ginseng is called stigmasterol, and that from Korean ginseng is called β-sitosterol (Hou, 1977).

1.3.3. SUGARS AND CARBOHYDRATES:

The aqueous extract of ginseng root contains many different types of sugar or saccharides. Ginseng pectin,

a crude polysaccharide, is also isolated from ginseng root (Hou, 1977).

1.3.4. MISCELLANEOUS COMPOUNDS:

Other components such as organic acids, non-protein nitrogenous substances, amino acid and peptides, vitamins, minerals and trace elements and certain enzymes have also been isolated and characterized (Hou, 1977).

1.4. PHARMACOLOGICAL STUDIES OF GINSENG:

During the past decades, a rather broad spectrum of different pharmacological activities of ginseng has been reported in Korea, Japan, China, Russia and Bulgaria. With regard to the pharmacologically active principles of ginseng one must distinguish investigations which have been done with the total extract from such investigations in which more or less purified fractions of the extract or chemically defined individual constituents from ginseng were used. The situation is further complicated by the fact that not only extracts from ginseng roots, but also extracts from other parts of the plant have been used. Furthermore the composition of the total extract or individual fractions may vary due to the species of ginseng, the place of origin, methods of growth or culture, technique of extraction and various other factors. Most pharmacological investigations in the past have been performed with total extracts from

ginseng roots. The following pharmacological or biological activities are summarised from review articles published one to two decades ago (Anon., 1977):

1. Ginseng extracts can increase the capacity for physical work and intellectual performance.
2. Ginseng preparations administered during prolonged periods are reported to accelerate recovery from diseases. In this connection it is sometimes emphasized that ginseng preparations do not show any unwanted side effects and that they do not lead to tolerance.
3. Ginseng effects on the central nervous system are not easy to interprete. In the early papers, tonifying and stimulating as well as depressing effects on the central nervous system were described.
4. Ginseng effect on the cardiovascular system was also studied. The results were equivocal; both hypertensive and hypotensive effects were reported.
5. Gonadotropic actions of ginseng have been studied and are less ambiguous.
6. Influences of ginseng on metabolic functions, particularly on carbohydrate metabolism have been noticed. Ginseng was reported to reduce blood glucose level.
7. Anti-inflammatory effect is also attributed to ginseng.
8. The "adaptogenic effect" of ginseng refers to an increase of the reactivity under stress situations and is manifested as increase of the defensive forces of the organism.

Some of the activities mentioned above are pharmacologically not very well defined and may involve simultaneously several organ systems or functions of the body or may encompass various types of pharmacological actions. Sometimes the results reported are even contradictory. It appears therefore necessary to try to analyse the whole complex of ginseng activities regarding effects in single organs, tissues and cells. Such an analysis would be facilitated if it could be based on investigations with chemically defined constituents. With regard to the large number of chemical compounds with a diversity of structures which have been detected in ginseng , opposite actions or contradictory results are probably not surprising.

Based on these new approaches during the last decade, the mysterious cloud surrounding the myth of ginseng is finally beginning to get demystified and its intrinsic biological effects are described in modern biomedical terms. Most of the recognized pharmacological activities are itemized below.

1.4. 1. EFFECT OF GINSENG ON CENTRAL NERVOUS SYSTEM:

In early publications stimulating as well as depressing effects on the central nervous system were described (Anon., 1977). This discrepancy is partly explained through investigations using purified fractions of the extract. A crude saponin fraciton from ginseng leaves was shown to exert

CNS-depressant, tranquilizing and analgesic action (Saito et at.,1973). Simultaneously, it was found that a ginseng neutral saponin fraction from the ginseng root could act on the CNS too (Nabata et al.,1973). This neutral saponin fraction contains R_{b1} , R_{b2} , R_c , the CNS-depressant effect of which was revealed by its inhibitory effects on spontaneous and exploratory movements, as well as potentiation of hexobarbital effect. It was later revealed that it was ginsenoside R_{b1} that exerted the CNS-depressant action and R_{g1} that exhibited CNS-stimulating activity (Takagi, 1977).

1.4.2. EFFECT OF GINSENG ON CARDIOVASCULAR SYSTEM:

A similar ambiguous situation appeared with ginseng effects on the cardiovascular system. A few years ago it was shown that certain fractions from ginseng might cause an increase of blood pressure while other fractions exerted the opposite effect (Anon., 1977). This discrepancy was not explained until the work of Takagi et al.(1972b). They found that the ginseng neutral saponin fraction containing R_{b1} , R_{b2} , R_c caused a fall in arterial blood pressure, but it had no effect on heart rate and respiration. The neutral saponins did not alter the characteristic response to a series of stimuli, and the hypotensive response to the saponin fraction was not eliminated by atropine, diphenhydramine and propanolol. As this neutral saponin fraction caused the fall of arterial blood pressure it was suggested

to have ganglion blocking activity.

The saponin fraction containing R_{g_1} caused the blood pressure to fall transiently, diminished the heart rate and accelerated respiration (Takagi et al., 1972b).

The effect of ginseng on the isolated heart was also studied. Both early (Anon., 1977) and recent studies (Bae, 1978) showed that ginseng had an inotropic effect on the isolated heart.

1.4.3. EFFECT OF GINSENG ON SEX HORMONES:

Less doubtful are sex hormone-like activities of ginseng preparations. Some previous investigations have furnished evidence for the occurence of sex hormone-like compounds in ginseng. Gonadotropic effects was shown to be exerted by sterolglycosides isolated from ginseng (Karzel, 1977), and by thin-layer chromatography, estrone, estradiol and estriol were detected in the liposoluble fraction of ginseng extract.

1.4.4. EFFECT OF GINSENG ON INTERMEDIARY METABOLISM:

1.4.4.1. SUGAR AND LIPID METABOLISM

With ginseng effects on the endocrine system or with hormone like actions of ginseng, influences on metabolic processes may be connected. During the last few years, most of the works were devoted to this topic, particularly to the potential effects on carbohydrate and lipid metabolism.

In early publications antihyperglycemic effects of ginseng have been reported (Anon., 1977). Recently, Yokozawa et al. (Yokozawa et al., 1975; Yokozawa and Oura, 1976) found that ginseng saponin-rich fraction could reduce blood glucose slightly. This decrease in blood glucose was accompanied by a significant decrease in liver glycogen (Yokozawa et al., 1976). Simultaneously, hepatic lipogenesis was also shown, but the lipogenic capacity of epididymal adipose tissue was more sensitive than that of the liver to a maximal dose of ginseng extract. Accumulation of lipid in adipose tissue was observed. On the other hand, lipogenesis by these tissues was markedly influenced by the nutritional status of the animals. Fasting of the animals resulted in the reduction of lipogenesis (Yokozawa and Oura, 1976). Upon refeeding, lipogenesis returned to normal level. Summing up the sequential stimulating biological actions on lipid and sugar metabolism as a result of a single dose of ginseng extract in the rat, the authors suggested that ginseng extract turned the metabolic flow in the direction of lipogenesis by the conversion of sugar, i.e. a transformation from carbohydrate to lipid, and its translocation from liver to adipose tissue. The phenomenon was further evidenced by the increased activity of pyruvate kinase, the enzyme catalyzing the last step of glycolysis, in rats treated with ginseng (Yokozawa et al., 1979).

1.4.4.2. CHOLESTEROL METABOLISM:

Ginseng was shown to stimulate the biosynthesis of cholesterol in liver *in vivo* (Sakakibara *et al.*, 1975; Gommori *et al.*, 1976). However, no stimulation could be observed with *in vitro* liver slices (Gommori *et al.*, 1976). It seems possible that most of the ginsenosides would affect the cholesterol synthesis not directly but through certain intermediate pathways which might involve the action of some hormones.

In the synthesis of cholesterol, the rate-limiting step is the conversion of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) into mevalonate, which is catalyzed by HMG-CoA reductase (EC 1.1.1.34). It was found that ginsenoside R_{b1} was associated with the increased activity of HMG-CoA reductase (Ikehara *et al.*, 1978).

The excretion of injected 4-^{14}C-cholesterol into bile and feces was enhanced by crude ginseng extract (Yamamoto, 1973). As suggested by the authors, the activity of cholesterol 7α-hydroxylase, an enzyme for cholesterol degradation, might be increased after ginseng treatment. Since it was found that the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase had a similar diurnal rhythm with the level of corticosterone in plasma (Cheitfetz, 1968), these enzymes were supposedly induced by corticosterone. The relationship between ginseng and corticosterone was thus indicated.

1.4.4.3. PROTEIN SYNTHESIS:

Another kind of potential metabolic activity of ginseng preparations concerns the protein metabolism. The stimulating effects of ginseng extract on the liver nucleus, cytoplasm and serum protein were shown to occur in sequential order. The first phenomenon observed was a stimulation in the Mg^{++}-activated DNA-dependent RNA polymerase activity (Hiai et al., 1971). Then there was an increase in the rate of liver nuclear RNA synthesis (Oura et al., 1971). Substantial stimulation in the cytoplasmic polysomal RNA synthesis followed, thus elevating the hepatic polysome content and the protein synthetic activity (Oura et al., 1972a). Finally, synthesis of serum protein was gradually stimulated (Oura et al., 1972b). Serum protein synthesis was later confirmed (Han et al., 1973) and the stimulating action was due to ginseng saponin (Oura et al., 1975). Based on these studies, it was suggested that the action of ginseng extract exerted a stimulatory action on the RNA and protein biosyntheses.

It has been known that the activity of RNA polymerase is increased in liver nuclear preparation of rats treated with cortisol (Litwack and Singer, 1972). Therefore, it was assumed that an enhancement in the rate of RNA and serum protein by ginseng extract might be due to the elevated concentration of adrenocortical steroids. Such a possibility was contradicted by some other observations.

It is a well-known fact that tryptophan pyrrolase and tyrosine transaminase can be induced by corticosterone (Schimke and Doyle, 1970; Litwack and Rosenfield, 1973), but the two enzymes were not significantly affected by ginseng extract (Oura et al., 1972). Therefore, at present, no definite conclusion can be drawn.

1.4.5. ADAPTOGENIC EFFECT OF GINSENG:

A number of investigations performed during the last decades aimed at demonstrating the adaptogenic effects of the ginseng preparations. It could be shown, for example, that ginseng extracts increases the duration of running ability , or prolonged significantly the swimming time of rats (Karzel, 1977).

It is of interest that some ginseng activities are only demonstrable in stressed, impaired or injured animals. Therefore, various types of experiments were designed using animals submitted to biological, physical or chemical damages. It could be shown that pretreatment with ginseng saponin prolonged the time leading to clonic and tonic extensor convulsions induced by pentylenetetrazol and to death induced by strychnine (Nabata et al., 1973). Ginseng neutral saponins showed a significant antipyretic activity in preventing the development of fever induced by a pyrogen (Nabata et al., 1973). Significant inhibition of writhing induced by acetic acid was exhibited by ginseng

neutral saponins (Takagi et al., 1972a; Nabata et al., 1973) and ginsenoside F from ginseng leaves (Saito et al.,1973). Six methods were used in the study of ginseng root on recovery from exhaustion: Exploratory movement tests, hole cross test, rotating rod test, sliding angle test, spring balance test and rectal temperature test. Anti-fatique effects of ginsenoside R_{g1} were obvious in every test while neutral saponin fraction had no effect on recovery in the 6 tests (Saito et al., 1974).

Some investigations dealed with protective effects of ginseng against cold or heat exposure. Here again usually no influence of ginseng on normal animals kept at room temperature was demonstrable (Li and Li, 1973). Some workers (Sung and Chi, 1958) found that when control rats were under stress at high temperature, they would be either unable to move or show clonic convulsion. However, the ginseng-treated rats were relatively normal when under stress. Another parameter used to indicate protective ginseng effects in animals under hyperthermic stress is the adrenal ascorbic acid (Sung and Chi, 1958; Kim et al.,1970). In animals under normal temperature conditions ginseng did not have any effect on the adrenal ascorbic acid content. After exposure to cold or heat, however, ginseng caused at first a faster decrease and subsequently a fast normalisation of the adrenal ascorbic acid content, while in control animals, a further decrease of the values occurred (Kim et al.,1970).

These results indicate that ginseng facilitates the reaction to and accelerates the recovery from temperature stress by modulating adrenal steroidogenesis.

Similar antistress effects of ginseng preparations could in some cases be demonstrated in animals under stress treated with the adrenocortical hormones.

This leads to the potential effects of ginseng preparations on the endocrine systems or to homone-like actions of ginseng itself. The question has been put forward whether particularly the antistress activties of ginseng, or other actions of ginseng preparations are mediated via the adrenal cortex, or the adrenocortico-pituitary system. Some findings seem to point out an involvement of this system but others do not. It could be shown for example, that ginseng extracts had a stimulating action on glucocorticoid production, which was reflected by the hypertrophy of the zona fasciculata at the border of the zona reticularis (Kim et al., 1970); adrenalecto-mized rats lost their ability to withstand temperature stress even under the influence of ginseng (Karzel, 1977). However, in other experiments ginseng preparations exerted antistress activities also in adrenalectomized mice (Karzel, 1977). Other effects of ginseng preparation, such as stimulation of serum protein synthesis (Oura et al., 1972), reduction of blood glucose and glycogen content (Yokozawa et al., 1975) could not be shown to be mediated through

the adrenal glands. Nevertheless, a recent study of Hiai et al. (1979) revealed a dose-response relationship between ginseng saponin and rat plasma corticosterone level. A maximum increase of corticosterone was found at a saponin dose of 8 mg/100 g body weight. This study has reinforced the belief that ginseng stimulates the adrenocortical system. The present work followed the same line of thought and the ginseng effect on the pituitary-adrenal axis was therefore examined.

1.5. ADRENALCORTICAL HORMONES——GLUCOCORTICOIDS

1.5.1. PHYSIOLOGY

Adrenalcortical hormones are hormones released from the adrenal cortex. These hormones fall into 2 groups: glucocorticoid and mineralcorticoid. The major glucocorticoid components are cortisol and corticosterone.

The activity of the adrenal gland is under the trophic control of the pituitary. In response to appropriate stimuli, the anterior pituitary secretes adrenocorticotrophic hormone (ACTH) which in turn stimulates the adrenal cortex to synthesize and release adrenal corticoids. The reslease of ACTH is, in fact, controlled by another trophic hormone, or set of hormones, called corticotropin-releasing factor, or CRF (Saffran et al., 1955; Schally et al., 1960). These hormones are discharged in the median eminence and are carried to the anterior pituitary by a

venous network called the pituitary portal system.

Essentially, the stimulated adrenal cortex secretes adrenocortical hormones into the bloodstream. About 30% glucocorticoids in blood circulates as free steroid (Daughaday and Mariz, 1961). Of the remainder, 50% is bound to low affinity, high capacity carrier associated with albumin and 20% to a high affinity, low capacity globulin of 52,000 molecular weight called "transcortin" or CBG (corticoid - binding globulin) (Westphal, 1971). The unbound steroid is thought to be the biologically active component of the system (Thompson and Lippman, 1974). The steroid-protein complex, in turn, may serve a buffer capacity, supplying additional unbound steroid as either free steroid is removed or as conditions change the binding constants between steroid and protein. The steroid-protein binding is markedly sensitive to temperature. Steroid-protein binding in serum, for example, is decreased by increasing temperature in and about the normal range (Pegg and Keane, 1969).

Release of ACTH from the pituitary and response of the adrenal to ACTH is influenced by the level of circulating corticoids (Sayers and Sayers, 1947). There are two feedback mechanisms on stress-induced release of ACTH namely, the level-sensitive and the rate-sensitive control (Smelik and Papaikonomou, 1973). In the level-sensitive (delayed) control, inhibition of the system

depends on the intensity of the stimulus and the level of circulating glucocorticoid (Smelik and Papaikonomou, 1973). For the fast rate-sensitive feedback (Dallman and Yates, 1969; Zimmermann and Critchlow, 1969; Jones et al., 1972; Jones et al., 1974), the magnitude of the effect is proportional to the rate of rise of hormone concentration. As the rate of rise of hormone concentration becomes small, the effect progressively reduces and finally disappears.

1.5.2. EFFECTS OF GLUCOCORTICOIDS:

Glucocorticoids have a wide range of actions on target tissues. These actions are thought to be of restorative importance, permitting return of the organism to homeostatic equilibrium.

The actions of glucocorticoids are remarkably tissue specific and a set of changes produced in one tissue may be quite opposite to those produced in another tissue. Thus, in liver, adrenocortical hormones increase hepatic urea formation (Engel, 1950) as a result of rapid amino acid catabolism, increase uptake of amino acids , increase liver protein nitrogen (Clark, 1953) at the expense of protein depletion of other tissues, and increase liver RNA without changing DNA level (Silber and Porter, 1953). The increase in hepatic protein following corticoid administration is due, in part, to hormonal induction of a variety of enzymes important in gluconeogenesis, glycolysis, urea

formation, and amino acid catabolism. Induction of these enzymes is preceded by an increase in liver RNA nucleotidyl transferase, increased RNA polymerase (Barnabei et al., 1966), increased RNA turnover, and an increase in the absolute amount of RNA (Feigelson et al., 1962). Glucocorticoids strongly affect liver glucose metabolism and indeed, such effects are perhaps primary to their adaptive function. Glucocorticoids, in vitro, inhibit glycogen formation by liver (Seckel, 1940). However, in vivo this is overcome by gluconeogenesis both from amino acids and compounds such as lactate, pyruvate, and succinate (Lewis et al., 1940).

What holds true for liver, however, is not necessarily true for other tissues. While liver RNA and protein concentration are increased by corticoids, RNA in both thymus and spleen is decreased while protein is decreased in thymus but not in spleen (Hofert and White, 1968; Abraham and Sekeris, 1971). The marked involution of thymus and other lymphatic tissue upon glucocorticoid treatment dramatically demonstrates the tissue selectivity of corticoid actions. Involution of these tissues appears to be due to a suppression of DNA synthesis and an inhibition of mitosis (Stevens et al., 1965), resulting in marked decrease in lymphocytes in the peripheral circulation . The number of circulating eosinophiles also declines (Navarette and Petit, 1962), apparently owing to destruc-

tion of these cells in bone marrow (Greendyke et al.,1965). On the other hand, polymorphonuclear neutrophils increase (Nelson et al., 1952) despite depressed mitotic activity (Cardinali et al., 1964), probably as a result of increased cellular half-life.

Like aldosterone, glucocorticoids in small doses reduce sodium excretion and increase potassium excretion (Ingle et al., 1946). They increase gastric secretion and produce gastric ulcers (Loeb and Sternschein, 1973). Autonomic reactivity is potentiated, cardiac output is increased , circulatory responses to epinephrine are potentiated, and capillary resistance is increased (Yard and Kadowitz, 1972). In addition, glucocorticoids inhibit inflammatory reactions (Dougherty et al., 1973), a property which has led to widespread therapeutic use. They also inhibit antibody production, impair migration of phagocytic cells and impair phagocytosis itself (Balow and Rosenthal, 1973). As might be expected from this enumeration, resistance to infection is decreased.

Little is known about the direct effects of adrenocortical hormones upon the brain itself, although considerable clinical and experimental literature exists on behavioral changes in adrenal malfunction. Adrenal insufficiency in Addison's disease is accompanied by behavioral changes such as depression, apathy, instability, and apprehension , together with changes in EEG. The reverse case

of adrenal hyperactivity in Cushing's disease is accompanied by euphoria, and sometimes overt psychosis (Martin, 1976).

2. EXPERIMENTALS

2.1. CHEMICALS

(1,2,6,7-^{3}H)-Corticosterone was supplied by New England Nuclear Corporation, specific activity 82.3Ci/mmole . Corticosterone for the standard was purchased from Sigma Chemical Co., U.S.A.. Adrenocorticotrophin (Cortrophin Z) was a product from N.V. Organon Oss, Holland. All chemicals used were of analytical grade.

2.2. PHYTOCHEMISTRY OF GINSENG

2.2.1. PREPARATION OF A TOTAL GINSENG SAPONIN EXTRACT:

Three-year old untreated dry roots of Panax ginseng C.A. Meyer were purchased from the herbal stores. It was originated from South Korea. Total ginseng saponin fraction was obtained according to the procedures of Sanada et al. (1974). (Fig. 1).

Six hundred g of dry ginseng root were blended into small pieces in a Waring blender. The blended plant material was then macerated in 21. of methanol overnight, and refluxed with the same methanol at 60°C for 4 hr. The reflux procedure was repeated two times using a new portion of methanol. After reflux was completed, the hot methanol extracts were pooled, filtered, and concentrated to dryness in a rotary evaporator under reduced pressure at 50°C. The dried extract was dissolved in a small quantity of distilled water and partitioned

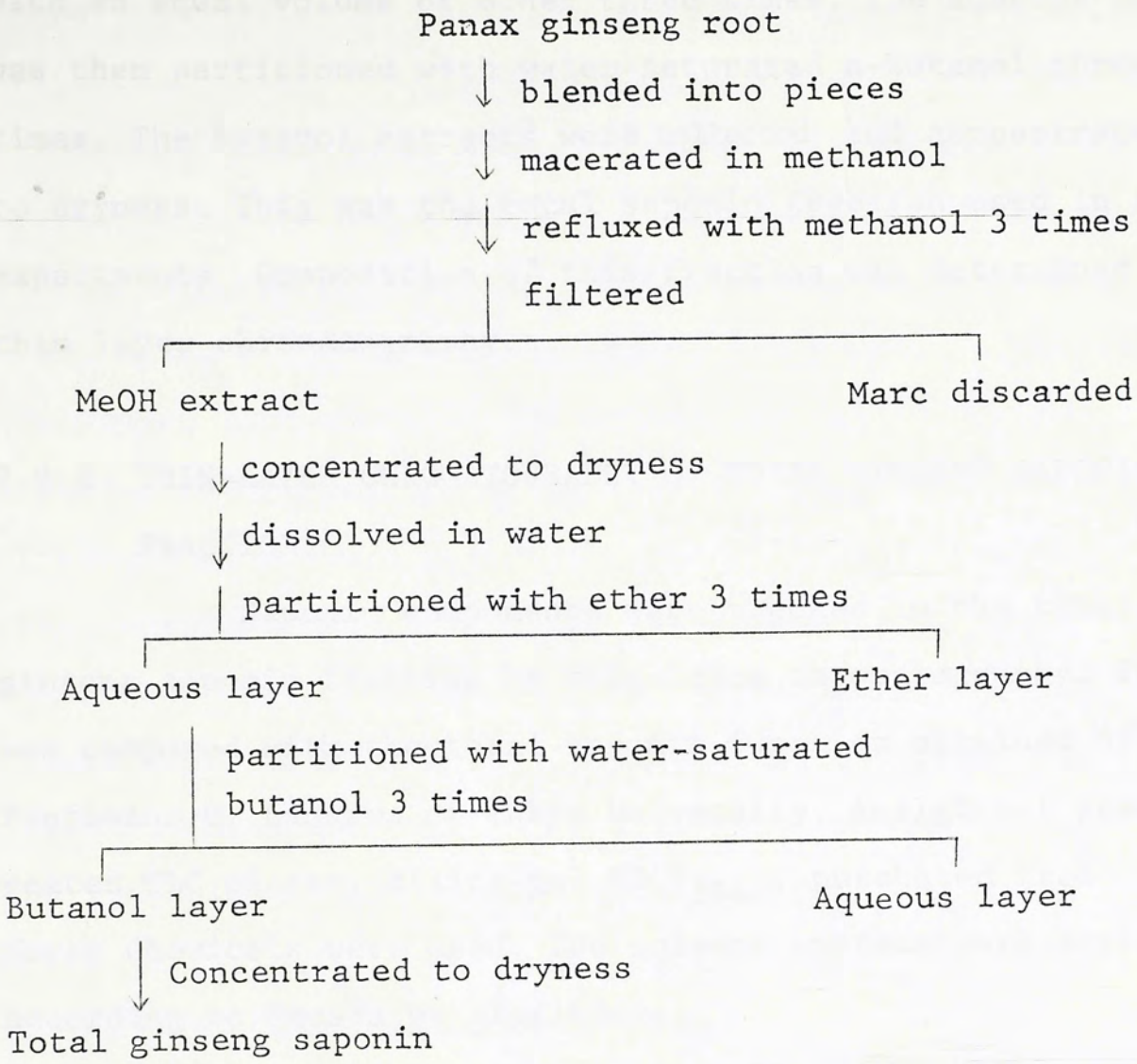


Fig. 1. Extraction of total ginseng saponin.

with an equal volume of ether three times. The aqueous phase was then partitioned with water-saturated n-butanol three times. The butanol extracts were collected and concentrated to dryness. This was the total saponin fraction used in the experiments. Composition of this fraction was determined by thin layer chromatography.

2.2.2. THIN-LAYER CHROMATOGRAPHY OF TOTAL GINSENG SAPONIN FRACTION:

Saponin components were checked in the total ginseng saponin fraction by thin-layer chromatography. It was compared with the total saponin fraction obtained by Professor U. Sankawa of Tokyo University. Analytical pre-coated TLC plates, silica gel 60 F_{254} , purchased from Merck Chemicals were used. Two solvent systems were employed according to Sanada et al.(1974):

(1) n-butanol:acetic acid:water (4:1:2)

(2) chloroform:methanol:water (65:35:10)

Pure crytalline R_g used as the standard was a gift from Professor U. Sankawa. The spots were detected by spraying with 10% H_2SO_4 and heating on a hot plate.

2.3. THE PREPARATION OF CORTICOSTERONE-BINDING GLOBULIN FOR COMPETITIVE PROTEIN BINDING ASSAY:

2.3.1. PREPARATION OF CBG:

Female albino Sprague Dawley rats of 200-250 g

were primed with estrogen 24 hr before sacrifice (single injection, i.m., 100ug). Blood was collected from the dorsal abdominal aorta. Blood samples were pooled together and allowed to clot in refrigerator. The serum so obtained was stripped to get rid of any corticosterone bound on the binding protein. For the stripping procedure, serum was incubated with charcoal (20 mg charcoal/ml serum) at 37°C shaking for 30 min. After incubation, the mixture was centrifuged at 7,000 x g (Sorvall RC2-B) at 4°C for 10 min. The supernate was distributed into small test tubes so that each tube contained 1 ml of serum sample. They were lyophilised and stored in a deep freezer. The dry serum was reconstituted and diluted to appropriate concentration with distilled water as the working solution.

2.3.2. DETERMINATION OF CBG TITER:

A fixed amount of tracer (40 nCi/ml corticosterone) was incubated with the binder (serum) obtained from above at increasing times of dilution 10, 30, 100, 300, and 1,000. The mixture was incubated first for 5 min at 45°C and then for 20 min in ice bath. Then 0.2 ml charcoal suspension (1% charcoal and 0.1% dextran) was added to separate the free and bound tracer. The mixture was centrifuged at 3,000 x g for 10 min, and the whole supernate was decanted into a counting vial containing 10 ml Triton-toluene scintillant (toluene 666 ml, Triton X-100 333 ml, PPO 5.5 g,

POPOP 0.1 g). Radioactivity was determined using the Beckman LS-330 Liquid Scintillation System, completed with background deduction and quenching control.

2.4. ANIMAL TREATMENT

Male rats of Sprague Dawley strain, weighing 160-190 g were housed in a light-controlled room (7:00-21:00 day cycle). They were fed with laboratory chow and water *ad libitum*.

Rats were divided into experimental and control groups. In the experimental group, animals were further divided into 2 subgroups——namely chronic group and chronic-acute group.

In the chronic group, animals were injected intraperitoneally 0.5 ml ginseng saponin in saline (10 mg total saponin fraction / 100 g body weight) for 2 days. Injections were made at 9:30 a.m. of each day. The rats were sacrificed on the third day.

For the chronic-acute group, the same treatment as the former group was followed except that the animals received one more injection on the third day morning one hour before sacrifice.

The control group received exactly the same treatment as the experimental groups except that the ginseng saponin fraction was replaced by the same volume of saline.

2.5. SAMPLE COLLECTION

Rats were sacrificed at 9:30-10:30 a.m. by guillotine. Trunk blood was collected in test tubes. The blood samples were allowed to clot in refrigerator overnight. Serum was separated and analysed for corticosterone by competitive protein binding assay according to the method of Pegg and Keane (1969). In the exsanguinated rat , the liver was perfused with cold isotonic saline via the hepatic portal vein. Pituitary, hypothalamus, cerebrum and livers were then removed. They were frozen immediately in liquid nitrogen and kept in a deep freezer. Pituitary, hypothalamus, and cerebrum were for the assays of corticosterone content which were done within 2 days, whereas the liver so collected was to be used for the determination of corticosterone binding capacity and affinity. The adrenal glands were also removed, trimmed of fat and kept in Krebs-Ringer bicarbonate solution (pH 7.4, 0.154 M NaCl, 0.154 M KCl, 0.11 M $CaCl_2$, 0.154 M KH_2PO_4 , 0.154 M $MgSO_4 \cdot 7H_2O$, 0.154 M $NaHCO_3$, containing glucose at 2 mg/ml) gassed with 95% O_2 + 5% CO_2 for 10 min at 37 C. Experiment on the adrenal response to ACTH was done immediately.

2.6. SAMPLE PREPARATION

2.6.1. PITUITARY, HYPOTHALAMUS AND CEREBRUM:

The pituitary, hypothalamus and cerebrum were homogenised with a Polytron type PCU-2 homogeniser in

dicholoromethane (3 ml for pituitary and hypothalamus, 10 ml for cerebrum). The tubes were kept in ice to prevent thermal inactivation. Homogenisation was continued until all the visible tissue particles were finely dispersed. The homogenate was then agitated on a Vortex mixer for 1 min and centrifuged in a clinical centrifuge for about 3 min; the tissue disc was removed by water suction. One ml aliquot of pituitary and hypothalamus extracts, and 3 ml of cerebrum extract were removed and placed in 12 x 75 mm culture tubes. They were dried at room temperature with a stream of nitrogen . The samples of pituitary, hypothalamus and cerebrum were then ready for the competitive binding assay of corticosterone.

2.6.2. SERUM:

A 0.2 ml aliquot of serum sample was added to 3 ml of dichloromethane and agitated on a Vortex mixer for 1 min. The serum layer was aspirated off. One ml of the organic layer was placed in a disposable culture tube and dried with a stream of nitrogen at room temperature. The serum sample was ready for the competitive protein binding assay of corticosterone.

2.6.3. LIVER:

Three g of liver were minced and rinsed in cold homogenization buffer solution (250 nM sucrose, 25 mM

KCl, 10 mM $MgCl_2$, 1 mM mercaptoethanol, in 50 mM Tris-HCl, pH 7.5). The minced tissue was homogenised in 6 ml buffer with Polytron type PCU-2 homogeniser and centrifuged at 105,000 x g for 1 hr in a Beckman Model L5-50 Ultracentrifuge at 4°C. The supernate was carefully removed and saved for the corticosterone binding assays. Protein determination was made by the method of Lowry et al (1951).

2.7. ASSAY METHODS

2.7.1. ASSAYS OF CORTICOSTERONE IN PITUITARY, HYPOTHALAMUS, CEREBRUM AND SERUM:

The content of corticosterone was estimated by the method of Pegg and Keane (1969) with modifications. The dried samples of pituitary, hypothalamus, cerebrum and serum were dissolved in 0.5 ml of 2% ethanol in 0.9% saline. The samples were agitated with a Vortex mixer to ensure complete dissolution. The binding reagent was prepared immediately before use by addition of 0.5 ml of tritiated corticosterone solution (4 uCi/ml) to 50 ml serum-CBG solution. A series of 0.5 ml standards were set up at concentrations 0.3, 1, 3, 10, 30 ng corticosterone/ml. A 0.5 ml aliquot of the binding reagent was added to the samples and standards. The tubes were shaken and warmed to 45°C for 5 min, then cooled in ice for at least 20 min. Then 0.2 ml of a dextran-coated charcoal suspension was added (1% aqueous suspension of Norit A from Serva, in 0.1%

dextran, M.W. 80,700) to separate bound and free steroids. The mixture was shaken and centrifuged immediately in Beckman Model TJ-6 Centrifuge with Model TJ-R Refrigeration Unit, at 3,000 x g 10 min. The whole supernate was decanted and mixed with 10 ml of scintillation cocktail in a counting vial. Radioactivity was determined using the Beckman LS-330 Liquid Scintillation System as mentioned above.

2.7.2. DETERMINATION OF BINDING CAPACITY AND AFFINITY OF THE BINDING PROTEIN IN LIVER :

The binding capacity and affinity were determined by the method of Pegg and Keane (1969) with slight modifications. Stripped liver homogenates were studied. Stripping was done by adding 0.2 ml charcoal suspension (20 mg / ml buffer) to 2 ml of liver homogenate. The mixtures were allowed to stand at 37°C for 15 min, and then centrifuged at 7,000 x g for 10 min. The supernates thus obtained will not contain the endogenous corticosterone bound on the binding protein.

Ten ul of various concentration of ^{3}H-corticosterone were mixed with 0.2 ml of liver homogenate to give a final concentration of 50, 25, 10, 5 ng corticosterone/ml. The samples were mixed and incubated at 4°C for 3 hr. The unbound steroid was adsorbed by addition of 0.1 ml dextran-coated charcoal suspension (3.75% charcoal, 0.375% dextran

in homogenisation buffer) and mixed. They were then centrifuged in a Beckman Model TJ-6 Centrifuge at 3,000 x g for 10 min. Then 0.1 ml supernates were counted in 10 ml of scintillation cocktail. The total redioactivity in the incubation mixture was determined by adding the homogenization buffer instead of charcoal.

Non-specific binding was estimated by preparing parallel solutions containing 500-fold excess of unlabeled corticosterone, following the same procedures as above. The difference in radioactivity (cpm) bound to cytosol proteins in the absence and presence of the 500-fold concentrated corticosterone solution was taken as an index for the number of the high affinity saturable binding sites for corticosterone.

2.7.3. DETERMINATION OF BINDING CAPACITY AND AFFINITY OF CBG IN SERUM:

Stripped serum samples were prepared by mixing 2 ml of diluted serum with 20 mg of Norit A. The mixture was incubated at 37°C for 30 min and then centrifuged at 7,000 x g for 10 min. The supernate containing the CBG was free of endogenous corticosterone. Serum had been diluted 5-fold with Tris buffer (0.05 M Tris-Cl, 0.1 M NaCl, 0.1% NaN_3 and 0.1% gelatin, pH 8.0). Ten ul of various concentration of ^{3}H-corticosterone were added to 0.2 ml of the serum samples to give a final concentration

of 200, 100, 50, 25, 10 ug/ml. The mixtures were allowed to stand for 5 min at 45°C and 5 min in ice bath. Then 0.1ml of dextran-coated charcoal suspension was added (3.75% charcoal, 0.375% dextran in Tris buffer). The suspensions were shaken , then allowed to stand for 10 min and centrifuged at about 3,000 x g for 10 min. Aliquots of the supernates were counted.

Non-specific binding was estimated as in liver homogenate. The difference between radioactivity measurements with and without the excessive unlabeled steroid represented the specifically bound corticosterone.

Both the binding capacity and binding affinity were obtained from analyses using the Scatchard plot (Scatchard, 1949).

2.7.4. EFFECT OF GINSENG SAPONIN ON ADRENAL RESPONSE TO ACTH:

Pooled samples of adrenal quarters obtained from control and ginseng-treated rats were used . Two quarters were taken in random as a set and mixed with 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.4 containing 2 mg/ml glucose in a polyethylene tube. The tube was gassed with 95% O_2 + 5% CO_2 , then capped and incubated at 37°C for 1 hr with gentle shaking. The incubation medium was decanted. Another 1 ml aliquots containing 0, 0.01, 0.1, 1 U of ACTH were added to the adrenal quarters and the procedure was

repeated. The incubating solution from each set was removed and assayed for corticosterone.

Corticosterone in the incubation medium was assayed by the method of Zenker and Bernstein (1958). The incubation medium samples were shaken vigorously with 3 ml of dichloromethane, and centrifuged at 3,000 x g for 2 min. The upper layer (aqueous phase) was aspirated off. The lower layer (CH_2Cl_2 phase) was then collected and shaken with an equal volume of conc. H_2SO_4 in absolute ethanol (H_2SO_4:EtOH, 7:3) for at least 15 sec. After standing for 30 min, the top layer of $CH_2Cl_2$was aspirated off, and the fluorescence of the bottom layer was read in a Turner Model 430 spectrofluorometer (excitation 470 nm, emission 525 nm).

The standard curve was obtained by running 0-0.4 ug of corticosterone through the same procedure.

3. RESULTS

3.1. CHARACTERISTICS OF TOTAL GINSENG SAPONIN FRACTION:

Thin-layer chromatography was employed in characterizing the components of the total saponin fraction obtained through the procedures as described in "METHOD".

Pure R_g obtained from Professor U. Sankawa was used as the standard. As shown in Fig. 2, the chromatograms of our saponin sample and that obtained from Professor Sankawa showed a similar pattern. As the composition of the two saponin fractions were almost identical, the results obtained from the two sources were pooled.

3.2. CBG TITER:

A dilution curve was constructed in order to choose the appropriate dilution of the working serum—— the binder.

A series of diluted serum was prepared as stated in "METHOD". According to Chard (1978), the concentration of binder sufficient to bind approximately 50% of the tracer is suitable for doing binding assays. From Fig.3 , it was shown that a 1:300 dilution of the binder satisfied this condition and was thus employed for all binding assays.

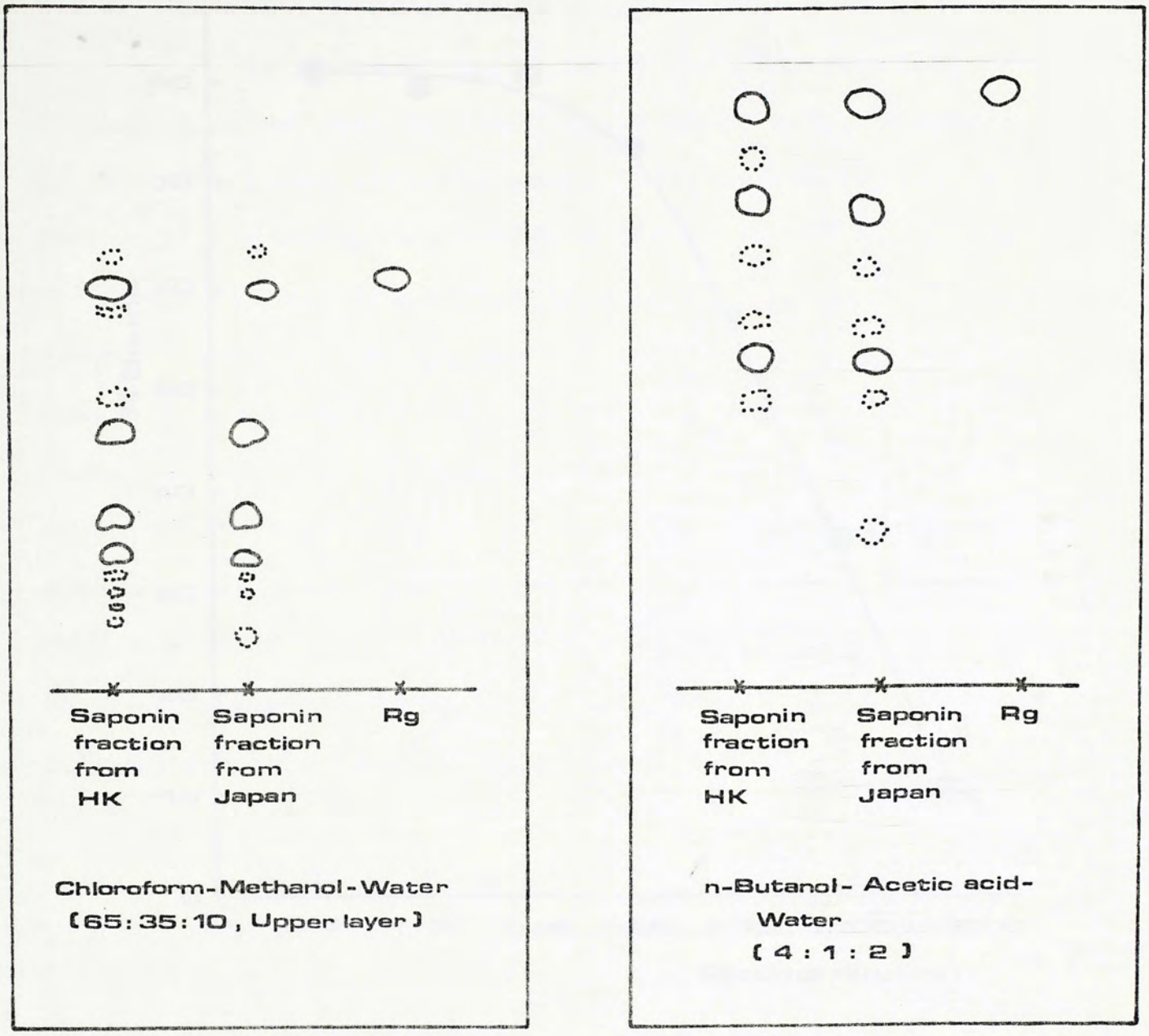


Fig. 2. Thin-layer chromatography of the total saponin fraction extracted from Panax ginseng

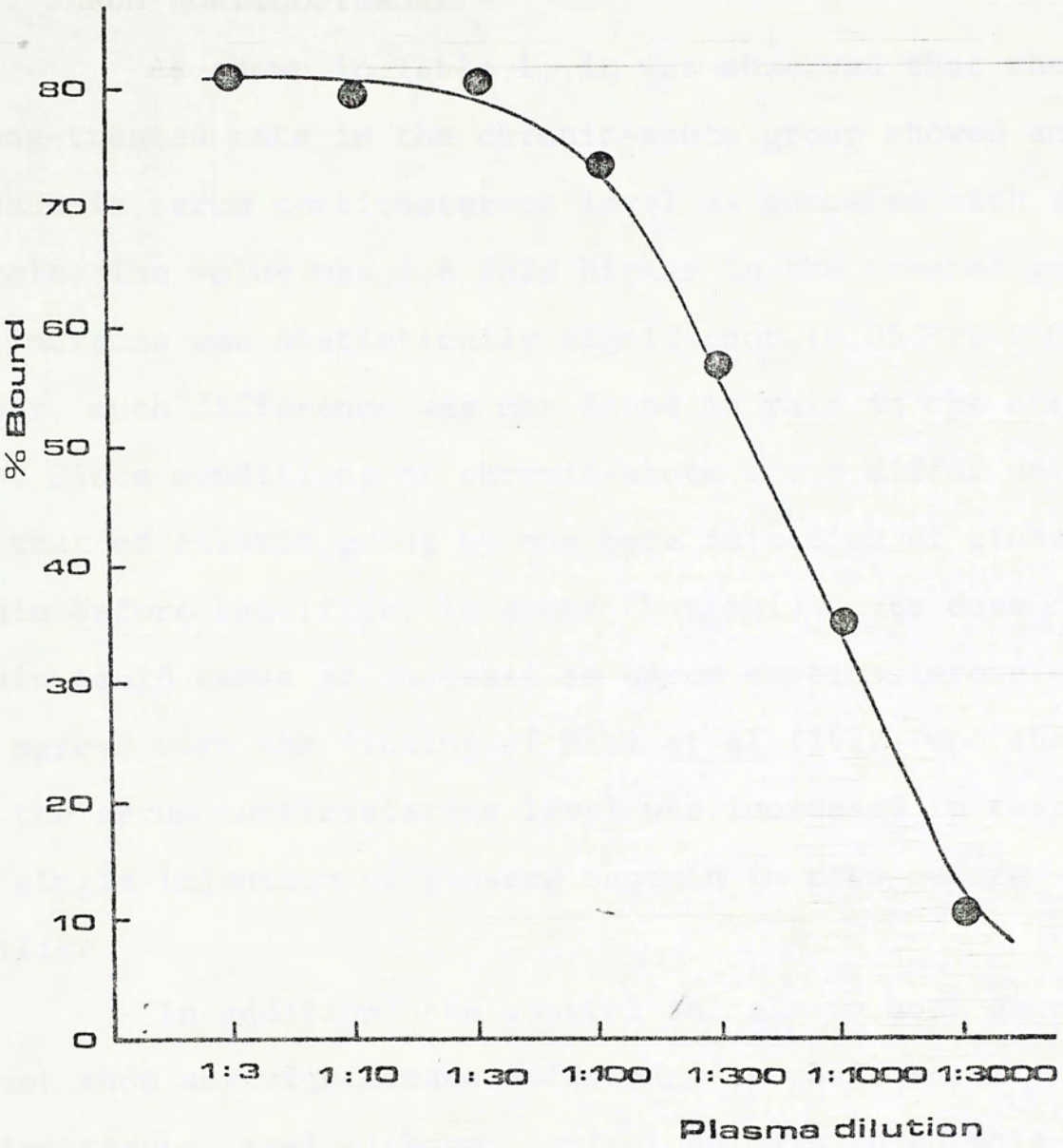


Fig. 3. A binder-dilution curve

3.3. CORTICOSTERONE LEVELS IN SERUM, PITUITARY, HYPOTHALAMUS AND CEREBRUM:

3.3.1. SERUM CORTICOSTERONE

As shown in Table 1, it was observed that the ginseng-treated rats in the chronic-acute group showed an increase in serum corticosterone level as compared with the controls. The value was 1.6 fold higher in the treated group. This increase was statistically significant ($0.05 > P > 0.01$). However, such difference was not found in rats in the chronic group. Since conditions of chronic-acute group differ only from that of chronic group by one more injection of ginseng saponin before sacrifice, it seems that this acute dose of saponin could cause an increase in serum corticosterone. This agreed with the finding of Hiai *et al* (1979) who showed that the serum corticosterone level was increased in response to a single injection of ginseng saponin in rats before sacrifice.

In addition, the control animals in both groups did not show any significant difference in their serum corticosterone level although control animals in chronic-acute group showed a slight increase.

3.3.2. CORTICOSTERONE IN PITUITARY

It is well established that pituitary is the site of feedback control for the secretion of ACTH (De Kloet and McEwen, 1976). Since ginseng saponin can increase the

Table 1. Effect of ginseng saponin on corticosterone level in rat serum.

Experimental group	Corticosterone (μg/100 ml)	Statistical difference
Chronic group		
Control	8.03±2.28 (10)	n.s.
Treated	9.86±1.52 (11)	
Chronic-acute group		
Control	12.45±2.96 (12)	$0.05 > P > 0.01$
Treated	20.59±2.55 (13)	

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean±S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means "not significant".
The difference between controls in both groups is insignificant ($0.4 > P > 0.3$).

level of corticosterone in serum, it would be interesting to determine the corticosterone level in the pituitary.

For rats in the chronic group, the ginseng treatment did not produce any significant difference in pituitary corticosterone level as compared with the control (Table 2). However, in the chronic-acute group, the ginseng treatment caused a 1.8 fold decrease in pituitary corticosterone level. Such a decrease was statistically significant ($0.05 > P \geq 0.01$). This was contradictory to the 1.6 fold increase in serum corticosterone level under the same condition.

Comparing the control values of corticosterone in Table 2, it was found that control in chronic-acute group had a slightly increased level of corticosterone than the control chronic group. This increase is not statistically significant. The elevation may be a response to the increased handling of the animals.

3.3.3. CORTICOSTERONE IN HYPOTHALAMUS

Corticosterone level in the hypothalamus was also determined. As shown in Table 3, no difference of corticosterone level was found in hypothalamus between control and treated rats in the chronic group. This was similar to the result in serum and pituitary.

However, for rats in chronic-acute group (Table 3), the difference between the ginseng-treated group and the

Table 2. Effect of ginseng saponin on corticosterone level in rat pituitary.

Experimental group	Corticosterone (μg/mg tissue)	Statistical difference
Chronic group		
Control	0.389±0.030 (5)	n.s.
Treated	0.368±0.019 (6)	
Chronic-acute group		
Control	0.499±0.050 (9)	$0.02 > P > 0.01$
Treated	0.276±0.066 (10)	

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean ± S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means "not significant".

Table 3. Effect of ginseng saponin on corticosterone level in rat hypothalamus.

Experimental group	Corticosterone (ug/mg tissue)	Statistical difference
Chronic group		
Control	0.089±0.003 (5)	n.s.
Treated	0.088±0.002 (6)	
Chronic-acute group		
Control	0.132±0.027 (9)	n.s.
Treated	0.128±0.021 (10)	

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean ± S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means "not significant".

control was still insignificant. The corticosterone level in the treated group remained the same in spite of the elevated serum corticosterone level (Table 1).

3.3.4. CORTICOSTERONE IN CEREBRUM

In order to study the ginseng action in the central nervous system, the corticosterone level in cerebrum was also determined (Table 4). Similar to the serum findings, there was no difference in cerebral corticosterone levels between control and experimental animals in chronic group. For rats in chronic-acute group, the ginseng treatment caused a marked increase in cerebral corticosterone ($0.01 > P > 0.005$). The 1.7 fold increase in cerebrum was equal in magnitude to the 1.6 fold increase in serum corticosterone.

When comparing the corticosterone levels in cerebrum of the two control groups in chronic and chronic-acute group, the latter had a much higher corticosterone level ($0.05 > P > 0.02$).

3.4. STEROIDOGENIC ACTIVITY OF ADRENALS:

In order to evaluate the effect of ginseng on the adrenocortical system against stress, the adrenal response to ACTH was studied *in vitro*. As shown in Fig.4a and 4b, both control and treated groups had typical dose-dependent response while adrenals of the ginseng-treated rats had an enhanced response to ACTH. Both ginseng-treated

Table 4. Effect of ginseng saponin on corticosterone level in rat cerebrum.

Experimental group	Corticosterone (μg/g tissue)	Statistical difference
Chronic group		
Control	12.51±5.58 (8)	n.s.
Treated	9.79±2.39 (11)	
Chronic-acute group		
Control	31.72±5.77 (9)	$0.01 > P > 0.005$
Treated	55.05±4.29 (9)	

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean ± S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means "not significant".

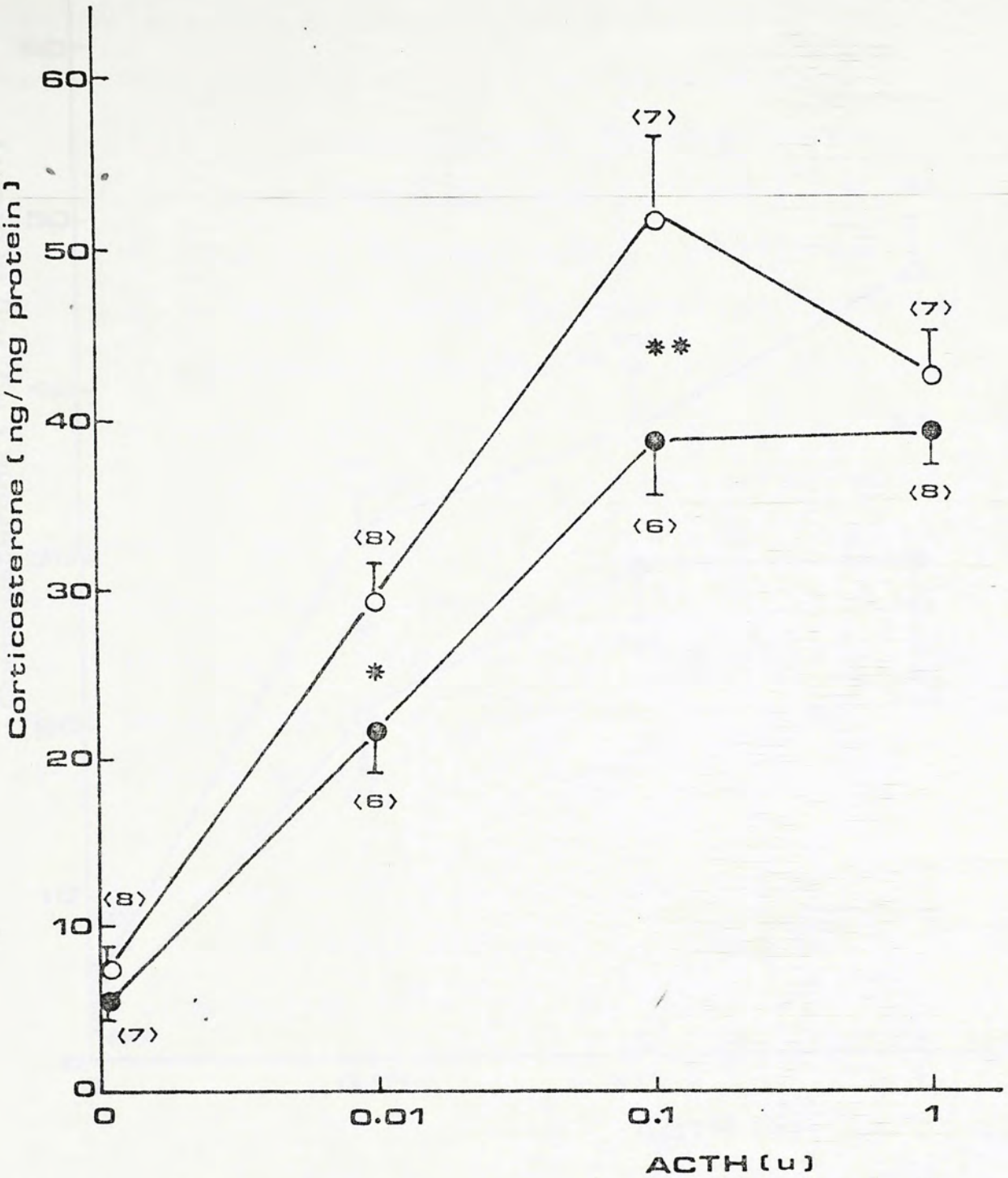


Fig. 4a. Effect of ginseng saponin on the adrenal response to ACTH in rats in chronic group.
The open circles represent the ginseng-treated rats, the dark circles are for the control rats.
Each value represents Mean ± S.E.
Numbers in parentheses indicate the number of adrenal samples.
* denotes $0.02 > P > 0.01$
** denotes $0.005 > P > 0.004$

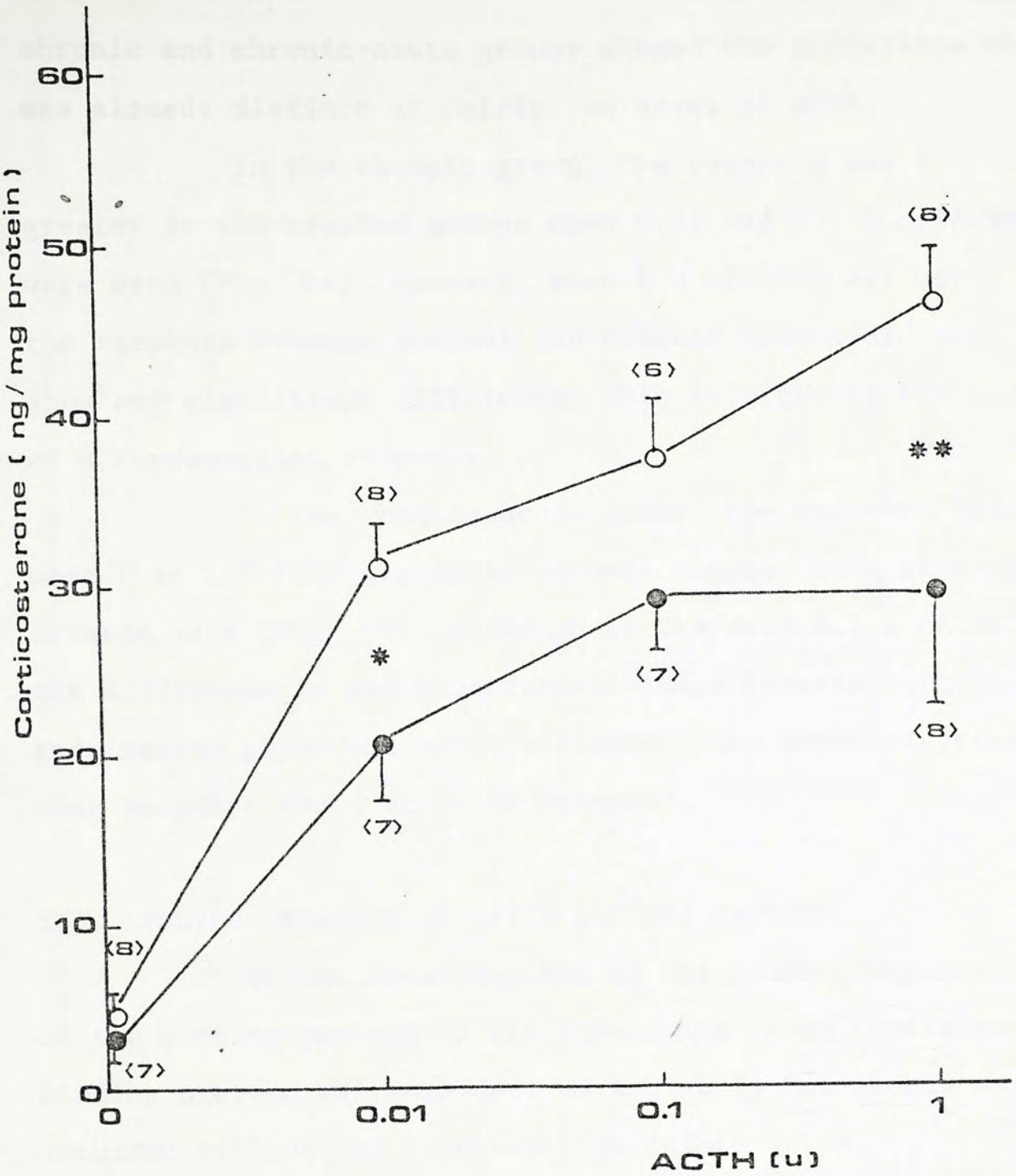


Fig. 4b. Effect of ginseng saponin on the adrenal response to ACTH in rats in chronic-acute group.
The open circles represent the ginseng-treated rats, the dark circles are for the control rats.
Each value represents Mean ± S.E.
Numbers in parentheses indicate the number of adrenal samples.
* denotes $0.02 > P > 0.01$
** denotes $0.0006 > P > 0.0005$

chronic and chronic-acute groups showed the difference which was already distinct at fairly low doses of ACTH.

In the chronic group, the response was 1.3 fold greater in the treated groups when 0.01 and 0.1 U of ACTH were used (Fig. 4a). However, when 1 U of ACTH was used, the response between control and treated groups did not show any significant difference. This is probably the result of a supramaximal response.

In the chronic-acute group, the response was more than 1.2 fold higher in adrenal samples from ginseng treated rats (Fig. 4b). Although at the dose 0.1 U of ACTH, the difference in corticosterone release between control and treated group was not significant, the trend of increasing response could still be observed.

3.5. BINDING CAPACITY OF LIVER BINDING PROTEIN:

In the investigation of the binding capacity of the binding protein in rat liver, the total corticosterone binding protein was regarded, as the whole homogenate was estimated without any fractionation before assay.

As shown in Table 5, the binding capacity was higher in the ginseng-treated rats in both chronic group and chronic-acute group. It could be noted that the increase in chronic group was 1.9 fold and the difference was significant. The increase in chronic-acute group was 1.7 fold, but for the large standard error in the treated group, the

Table 5. Effect of ginseng saponin on binding capacity of liver binding protein.

Experimental group	Binding capacity (nM/mg protein)	Statistical difference
Chronic group		
Control	6.81±0.84 (6)	0.0003 > P > 0.0002
Treated	12.83±0.80 (8)	
Chronic-acute group		
Control	6.08±1.07 (6)	0.2 > P > 0.1 (n.s.)
Treated	10.12±2.23 (7)	n.s.

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean ± S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means "not significant".

difference between the treated and the control would not be significant. Nevertheless, the trend of increase was still apparent.

It should be noted that the control animals in chronic group and chronic-acute group had almost the same binding capacity; also the value obtained from the treated chronic group was close to that in the treated chronic-acute group.

The binding affinity was the same for control and treated animals in both groups. A value of $(1.77 \pm 0.42) \times 10^7\ M^{-1}$ was obtained.

3.6. BINDING CAPACITY OF SERUM CBG:

As shown in Table 6, there was no change in binding capacity of serum CBG in the ginseng-treated animals in both groups, indicating the concentration of CBG in serum was not increased after ginseng treatment. The binding affinity of serum CBG was similar for all groups . The association constant found was $(1.17 \pm 0.32) \times 10^7\ M^{-1}$.

Table 6. Effect of ginseng saponin on binding capacity of serum CBG.

Experimental group	Binding capacity (nM/ mg protein)	Statistical difference
Chronic group		
Control	30.72±6.24 (8)	n.s.
Treated	36.21±4.85 (11)	
Chronic-acute group		
Control	35.65±2.82 (7)	n.s.
Treated	32.35±3.45 (8)	

Chronic group and chronic-acute group were described in "METHOD".
Data are expressed in mean ± S.E.
Numbers in parentheses indicate the number of rats in each group.
n.s. means not "not significant".

4. DISCUSSION

4.1. CHOICE OF GINSENG SAMPLE:

Total ginseng saponin fraction was used in treating rats in every test. A total crude aqueous extract of ginseng had been tried but results were equivocal. Since the concensus of international literatures agree that the bioactive component in ginseng is saponin, total saponin fraction was used instead of total crude extract. It was noted that the effect of total saponin fraction is more consistent than that of the crude extract.

4.2. APPLICATION OF METHODS:

4.2.1. WAY OF SACRIFICE

Decapitation was chosen as the way of sacrifice for the rats because it is the quickest way to kill the animal. It has been found that (De Kloet and McEwen, 1976) ACTH secretion following a noxious stimulus is maximal within 2 min, with maximal corticosterone concentration observed after 15 min. Generally, basal rat plasma corticosterone level is 13.0 ± 1.2 µg/100 ml plasma; under ether stress, the plasma corticosterone level rises to 32.1 ± 1.2 µg/100 ml plasma in two min (Feldman, 1974). Therefore ether anesthesis was not used in sacrifice in case the high level of corticosterone would mask the effect of ginseng saponin.

The corticosterone secretion following a stressful stimulus (such as handling) applied during the diurnal trough peaks at 5 min after application and then falls rapidly. When applied during diurnal crest, the same stimulus caused a much slower adrenocortical activation, reaches a maximum after 30 min, and decreases slowly (Alder and Friedman, 1968). However, if decapitation is the way of sacrifice, the animal will die before the adrenals can manage to release corticosterone.

4.2.2. TIME OF SACRIFICE

The time chosen for sacrifice of rats was 9:00 to 10:00 in the morning. This time interval is within the range in which the corticosterone level is the lowest in the circadian rhythm of the day (Stevens et al., 1973). If the animal is sacrificed by the time when the corticosterone reaches a peak, then the effect of ginseng on the corticosterone level will be mitigated. Moreover, the corticosterone level between 8:00 and 12:00 a.m. is a plateau, hence less fluctuation is encountered.

4.2.3. CBG AS A BINDER

Serum CBG was applied as a binder in the competitive protein binding assay because it possesses a number of advantages (Murphy , 1971). Firstly it is easiest to obtain and prepare. Usually one needs merely to obtain

blood by venipuncture, centrifuge it to obtain serum, and dilute the serum to an appropriate concentration. Secondly, it is very stable. If it is stored frozen in deep freezer, it keeps for years, and if it is refrigerated, it keeps for three weeks or often much longer. One big advantage of the naturally-occuring proteins over antibodies to hormone conjugates is the stability within a given species. With antibodies, even from the same animal, the titer and specificity of every batch is different. As for CBG, its properties within any given species are consistent so that data about its binding properties are comparable from experiment to experiment.

4.2.4. TITER OF CBG

In the competitive protein binding assay, a 1:300 dilution of the binder (serum CBG) was employed and an appropriate amount (50%) of the tracer was bound at such dilution (Fig. 3). In other studies (Pegg and Keane, 1969; Gould and Siegel, 1978) , a 25-fold dilution of the binder was employed. It may be due to the fact that the binder used in the present experiment was obtained from female rats primed with estrogen one day before the blood was collected. It is a well established fact that the CBG binding capacity of female rats treated in this manner is much higher than normal male rats without treatment (Westphal,1971). Therefore it can be reasoned that the CBG

obtained in this way has a titer much higher than those obtained without estrogen pretreatment .

4.3. ACUTE AND CHRONIC EFFECT OF GINSENG SAPONIN ON THE ORGANISM:

From previous studies, protein synthesis in liver (Han et al., 1973) and serum (Oura et al., 1972) had a slight increase after an acute dose of ginseng saponin. Such an acute dose could also increase the serum corticosterone level in rats (Hiai et al., 1979). The chronic effect of ginseng has not been tested. Traditionally people used to take ginseng over a prolonged period of time. This indicates that ginseng should have a long-term effect. The question whether chronic doses of ginseng could have more contribution is considered in this study.

In the present study, the chronic effect of ginseng was investigated by treating the experimental animals with 2 doses of ginseng with the last dose given at 24 hours before sacrifice (the chronic group). It was found that the serum corticosterone was not increased under such treatment. Also, pituitary, hypothalamus and cerebrum had no change in corticosterone level too. However, the steroidogenic activity of the adrenal was enhanced and the binding capacity of the liver binding protein was increased.

while the experimental animals were treated as above but with one more injection one hour before sacri-

fice (the chronic-acute group), all the parameters showed appropriate changes , the serum and cerebrum showed an increased level of corticosterone, the pituitary showed a decreased corticosterone level, the adrenals showed a better response, and the binding capacity of liver binding protein was augmented.

From the data obtained , it can be deduced that ginseng effect on serum corticosterone level is acute. This is good for sudden, unexpected severe stress, in which case ginseng may immediately serve as an anti-stress agent.

While the acute effect of ginseng can be demonstrated by the serum corticosterone level, the chronic effect of ginseng can be revealed from the steroidogenic activity and the binding capacity of the liver binding protein.

As mentioned above, the adrenals of both treated groups (chronic group and chronic-acute group) had a better response to ACTH than the control groups. This finding is consistent with the work of Kim et al.(1970) who found that the adrenal ascorbic acid depletion in response to ACTH was faster in the rats treated with ginseng than the control. It should also be noted that one more injection before sacrifice did not cause any additional difference in the response of the adrenals to ACTH. This shows that ginseng is unable to produce these changes in

one hour but these effects can persist for quite a long period of time.

Another support to the chronic effect of ginseng saponin is evidenced by the increased binding capacity of the corticosterone binding proteins in liver. In the present study, the whole liver cytosol was tested and individual binding proteins were not separated. According to literatures, there are at least seven binding proteins present in the liver (Beato et al., 1972a; 1972b ; Beato and Feigelson, 1972a; 1972b; Litwack et al., 1973; 1975 ; Feigelson et al., 1978). It is impossible to conclude which binding protein has been increased after ginseng treatment. Nevertheless, it is certain that the binding proteins tested do not include binder I and binder III found by Litwack et al.(1973), because the ligand used in the assay was tritiated corticosterone and not its metabolites. Binder I and binder III bind the metabolites of corticosterone. Also, the binding proteins tested probably do not contain protein G as found by Beato et al (1972b) because this protein is very labile. Therefore the liver homogenate tested in the present study may contain transcortin, protein B (Beato and Feigelson, 1972a), Binder II (Litwack et al., 1973), and binder IB (Litwack et al., 1975). Since at the present moment, the exact role played by each binding protein is still unknown, it is not attempted to check which type(s) of protein were involved.

The present results show that the binding capacity of the binding protein in liver is higher in the ginseng-treated rats in both chronic and chronic-acute groups without any change in binding affinity. This increase in capacity might be due to de novo synthesis of more binding proteins, as it is well established that ginseng stimulates liver protein synthesis (Han et al., 1973). As it has been reported, the anabolic effect of corticosterone can be demonstrated in terms of the enhanced de novo synthesis of a small group of inducible enzymes (Schimke and Doyle, 1970), these inducible enzymes function mainly in the catabolism of amino acids and are typified by tyrosine aminotransferase and tryptophan oxygenase (Litwack and Rosenfield, 1973). According to our preliminary observation, the increase in binding protein might also be involved in slight change in enzyme activity of tyrosine aminotransferase and tryptophan oxygenase. However, the information at hand is too limited to afford any conclusion.

4.4. EFFECT OF GINSENG SAPONIN ON SERUM CORTICOSTERONE:

From the present data, there was no observable change in serum corticosterone in rats sacrificed 24 hours after the last injection. For rats following the same schedule but treated with one more dose of ginseng saponin just one hour before sacrifice, significant increase of serum corticosterone level was found. This implicates

that ginseng saponin can only cause a transient increase of corticosterone concentration in serum that will not persist at the elevated level for up to 24 hours. In normal rats, there is a diurnal variation in serum corticosterone throughout the day (Stevens et al 1973). This increased level caused by ginseng saponin is almost comparable to the peak value of the diurnal rhythm, which is still within the physiological range. However, it seems that rats do not maintain such a high level of corticosterone for longer periods of times, as demonstrated from the data in the chronic group, otherwise they might suffer from hyper-corticosteronism.

It was also found that the serum level of corticosterone binding globulin (CBG) did not show any change following the administration of ginseng saponin (Table 6). Therefore, majority of the increased corticosterone will be in free form rather than being bound to globulin. Since the free corticosterone has a half life of about 20 minutes (Schapiro et al, 1971), it is not surprising to expect the fall of corticosterone level after a few hours unless the adrenals sustain an increased output of corticosterone. But in this case, the body will suffer from hypercorticosteronism, such a phenomenon caused by ginseng has never been reported in previous literatures. But recently, adverse effects due to abused use of ginseng preparations are known to occur. Hence,

the assumption that ginseng only causes a transient increase of corticosterone seems to be plausible. Moreover, rats receiving 4 daily doses of ginseng saponin and sacrificed on the fifth day have no increase in serum corticosterone (data not presented). However, more experiments should be carried out to see how the serum corticosterone declines after the rise in response to an acute adminstration of ginseng saponin.

4.5. EFFECT OF GINSENG SAPONIN ON CBG:

It is well established that the serum proteins are synthesized in liver (Westphal, 1971) and released into the plasma. From the data of the liver binding protein (Table 5) , there is an increase in the binding capacity, indicating that the protein content is increased in the liver. But according to the data of the serum CBG (Table 6), there is no change in the binding capacity. Therefore the increased protein in the liver may not be an increase in absolute amount of transcortin, it may be an indication of the increased turnover rate of CBG.

From another point of view, CBG increase may not be necessary if the assumption that serum corticosterone level only shows a transient increase is correct. It has been reported that CBG bound corticosterone is biologically inactive (Thompson and Lippman, 1974).

Therefore, if the serum corticosterone remains unchanged and serum CBG is increased, then the available free corticosterone will be decreased relatively. This will not benefit the regulatory system of the organism.

4.6. EFFECT OF GINSENG SAPONIN ON PITUITARY CORTICOSTERONE:

From the present data, a reciprocal relation is found between the serum corticosterone level and pituitary corticosterone level. The result shows that as the serum corticosterone increases, there is a decrease in pituitary corticosterone . This is not consistent with the usuall feedback mechanism (Ganong, 1978). The discrepancy can be explained by assuming that the ginseng saponin may possibly compete with corticosterone for the binding sites on the pituitary, so that pituitary corticosterone is displaced by the ginseng saponin. Although preliminary experiments showed that ginseng saponin did not compete with corticosterone for the binding sites on pituitary *in vitro* (data not presented), this may be due to the fact that the saponin fraction used in the experiment was not pure enough. Competitive protein binding experiment using isolated saponin was not attempted. Therefore, the possibility of a displacement at the pituitary level always exists.

As a matter of fact, the decrease in pituitary corticosterone level in turn can stimulate the release

of CRF from hypothalamus, the CRF is transported by the portal vessels to the anterior pituitary (McCann and Porter, 1969; Ganong, 1977). Special cells in the anterior pituitary release ACTH into the vascular system and ACTH stimulates subsequently adrenal glucocorticoid secretion, which will result in a better response of adrenal to stimulus. Taken together, the decrease in pituitary corticosterone level, the better adrenal response to ACTH challenge, and the increase in liver corticosterone binding protein favor the synthesis and release of glucocorticoid and are thus helpful to the body to resist stress.

4.7. EFFECT OF GINSENG SAPONIN ON HYPOTHALAMUS CORTICOSTERONE:

In the late 1960's, it was accepted that the localization of glucocorticoid receptors is mainly in the hypothalamic area, where the CRF is stored (Chowers et al., 1963; Corbin et al., 1965). Electrical or mechanical stimulation of the hypothalamic area results in the discharge of ACTH, while lesions can practically inhibit the ACTH secretion (Mangili et al., 1966). The binding of corticosterone in the hypothalamic region is thought to be related to the role of these hormones in negative feedback mechanisms involved in the regulation of ACTH secretion (Ganong and Hume, 1954). Other studies show that destruction or removal of the hypothalamus does not interfere with the feedback

control of ACTH release (De Wield et al., 1964; Kendall et at., 1964). These show that the hypothalamus may or may not be essential for the feedback action, at least in the suppression of stress-induced ACTH release by supraphysiological levels of glucocorticoids. However, studies of ^{3}H-corticosterone distribution in the brain reveal that there is a distinct regional difference in the uptake of the radioactive steroid. The highest uptake is, however, not observed in the hypothalamus (McEwen et al., 1972; Knizley, 1972).

In the present study, the results show that ginseng saponin has no effect on the hypothalamus corticosterone level both in the chronic group and the chronic-acute group, in spite of an increase in serum corticosterone level of up to 25 µg/100 ml. It can be deduced from the above investigations that hypothalamus may not be closely involved in the feedback mechanism. Nevertheless, for the time being, it is too premature to conclude that the hypothalamus is not a site of action of ginseng saponin. Furthermore, since the hypothalamus is not a well-defined area in the brain, there is a possibility that there might be some experimental error in obtaining the exact anatomic region every time. Additional studies will be required for confirmation.

4.8. EFFECT OF GINSENG ON CEREBRUM CORTICOSTERONE:

In the cerebrum, when the chronic control group is compared to the chronic-acute control group, a significant increase in the corticosterone level in the chronic-acute group is observed. This increase may somehow arise from both handling and injection, which can cause an increase in corticosterone release from the adrenals and bound to the cerebrum.

In a study of diurnal variations in brain capacity, it was found by Stevens et al.(1973) that the amount of ^{3}H-corticosterone bound by brain protein was inversely proportional to the circulating level of corticosterone. This substantiates an earlier report (McCann and Porter, 1969) that increased adrenocortical secretion leads to an increase in the amount of corticosterone that can be extracted from the brain. Therefore in the present study, it is quite logical to find a parallel increase in cerebrum corticosterone level corresponding to the elevated serum corticosterone level in the chronic-acute group.

In the cerebrum, the hippocampus is responsible for most of the corticosterone binding. Studies of ^{3}H-corticosterone distribution in the brain reveal that the highest uptake is observed in the hippocampus (McEwen et al., 1969; 1972b; Knizley, 1972). The largest amount of binding protein is also found in the hippocampus (McEwen

et al., 1972a) and it has therefore the highest binding capacity.

The physiological roles of corticosterone in the hippocampus have been studied. It is found that stimulation of hippocampus provokes the blockade of ACTH release under basal and stress conditions (Mangili et al., 1966; Kawakami et al., 1968). Along with serveral other brain regions, the hippocampus has been shown to be a target area for subtle effects of corticoids on ACTH secretion. These subtle effects involve elevations or reductions of basal levels of plasma corticosterone without necessarily blocking ACTH secretion (De kloet and McEwen, 1976). Therefore, the increased corticosterone level in cerebrum (the site of action is regarded to be the hippocampus) of the treated rats may contribute to the inhibition of the corticosterone release. Whether some of these effects may involve indirect actions via interaction of corticosterone with receptors in hippocampus, septum and amygdala remains to be investigated (McEwen et al., 1972; De Kloet and McEwen, 1976).

It is reported that the increased corticosterone in the hippocampus is said to have important effects on neural processes underlying behavior in rats (Bohus, 1973). It will not be surprising to find that such an increase is also related to the emotional and vegetative responses of the organism. However, whether ginseng has

any interrelationship with these effects will require more investigations.

4.9. GENERAL DISCUSSION:

Stress is a very complicated physiological reaction. Upon stress, the regulating system may involve co-operation of various hormones, among which, glucocorticoids play the decisive role. Mortality in experimental animals suffering from severe stress is obvious. Animal studies have revealed that ginseng can reduce mortality of the rats exposed to stress (Karzel, 1977; Anon., 1977).

From previous studies, ginseng was shown to increase 17-ketosteroids in urine (Petkov and Staneva-Stoicheva, 1965). It could also facilitate the depletion and recovery of ascorbic acid in the adrenal (Kim et al., 1970). The above phenomena are confirmed in the present study by testing the corticosterone in serum directly. Reduced amount in the pituitary suggests that short loop inhibition of ACTH release is removed. These two parameters are not affected when injection of ginseng is given 24 hours ago. This may indicate a transient increase of corticosterone after ginseng administration and may reflect an acute effect of ginseng. The long-termed effect of ginseng is demonstrated in the increase of liver binding proteins as well as the augmentation of steroidogenesis upon ACTH stimulation with or without pre-sacrifice ginseng

injection. In the light of these observations, ginseng may possess both acute and chronic function, at least in this particular site of action. Influence of ginseng on the corticosterone content in the central nervous system may involve modification of behavior. Studies on this topic, although recurrent, are still ambiguous and uncertain.

The precise manner in which ginseng saponin can regulate the body and protect it against stress may well be complex. It is therefore unwise to draw far-reaching conclusion from the limited data presented here. The increased glucocorticoid level that accompanies stress is sure to play an essential role in survival. To what extent the biological effect of ginseng is acted through glucocorticoid is difficult to quantitate. Hypophysectomy and adrenalectomy seem to be the next logical step in the study of ginseng effect on pituitary-adrenal axis. The long-termed effect of ginseng on the kinetics of circulating corticosterone level throughout the diurnal rhythm awaits further examination. Investigations using individual saponin components will definitely give more meaningful answers.

5. SUMMARY

1. Rats were divided into chronic group and chronic-acute group . Ginseng saponin fraction was shown to exert no effect on serum corticosterone level of the chronic group. But a 1.6 fold increase in serum corticosterone was found in the ginseng treated chronic-acute group. This may indicate a transient increase of serum corticosterone after acute ginseng treatment.
2. The pituitary corticosterone level was the same in control and ginseng-treated chronic group, while a 1.8 fold decrease in corticosterone level was shown by the ginseng treated chronic-acute group. This will lead to an increase in the release of corticosterone in the adrenals.
3. The hypothalamic corticosterone level was not affected in all ginseng-treated groups.
4. Ginseng chronic treatment did not affect the cerebrum corticosterone level. However, parallel to the serum corticosterone, a 1.7 fold increase in cerebrum corticosterone was exhibited by chronic-acute ginseng treatment.
5. The steroidogenic activity of the adrenals under the stimulus of ACTH was enhanced in both ginseng-treated chronic and chronic-acute groups, indicating the long-termed effect of ginseng on the organism.
6. The capacity of the liver binding proteins was increased in both ginseng treated-groups, indicating the increased

concentration of binding proteins. The exact type of binding protein was not determined. The binding affinity was the same for control and treated animals in all groups.

7. The capacity of serum CBG was the same for control and ginseng-treated animals in chronic and chronic-acute group. The binding affinity did not show any change in all animals.
8. The acute effect of ginseng could be demonstrated by the increased serum corticosterone level, while the long-termed effect of ginseng could be exhibited by the enhanced steroidogenic activity in the adrenals and the increased binding capacity of liver binding proteins.

6. REFERENCES

Abraham, A.D. and Sekeris, C.E. (1971) Inhibitory Action of Steroid Hormones on RNA Synthesis of Isolated Thymus Nuclei. Biochim. Biophys. Acta 247: 562-569

Alder, R. and Friedman, S.B. (1968) Plasma Corticosterone Response to Environmental Stimulations: Effects of Duration of Stimulation and 24-hour Rhythm. Neuroendocrinology 3: 378-386

Anonymous (1977) Encyclopedia of Chinese Medicine. PP. 29-36, People's Press, Shanghai, People's Republic of China.

Bae, H.W. (1978) Korean Ginseng, 2nd Edition, Korean Ginseng Research Institute, Korea.

Balow, I.E. and Rosenthal, A.S. (1973) Glucocorticoid Suppression of Macrophage Migration Inhibitory Factor. J. Exp. Med. 137: 1031-1039

Barnabei, O.,Romano, B., DiBitonto, G. and Tomasi, V. (1966) Factors Influencing the Glucocorticoid-induced Increase of Ribonucleic Acid Polymerase Activity of Rat Liver Nuclei. Arch. Biochem. Biophys. 113: 478-486

Beato, M. and Feigelson, P. (1972) Glucocorticoid-binding Proteins of Rat Liver Cytosol. J. Biol. Chem. 247: 7890-7896

Beato, M., Kalimi, M. and Feigelson, P. (1972) Correlation Between Glucocorticoid Binding to Specific Liver Cytosol Receptors and Enzyme Induction in vivo. Biochem. Biophys. Res. Comm. 47(6): 1464-1472

Beato, M. Schmid, W. and Sekeris, C.E. (1972) Two Cortisol Binding Proteins from Rat Liver Cytosol. Biochim. Biophys. Acta 263: 764-774

Bohus, B. (1973) Pituitary Adrenal Influences on Avoidance abd Approach Behaviour of the Rat. In "Drug Effects on Neuroendocrine Regulation, Progress in Brain Research", edited by E. Zimmermann, W.H. Gispen, B.H. Marks and D. DeWied, Vol. 39, pp. 407-419, Elservier, Amsterdam.

Cardinali, G., Cardinali, G., DeCaro, B.M., Handler, A.H. and Abonl-Enein, M. (1964) Effect of High Doses of Cortisone on Bone Marrow Cell Proliferation in the Syrian Hamster. Cancer Res. 24: 969-972

Chard, T. (1978) An Introduction to Radioimmunoassay and Related Techniques, edited by T.S. Work and E. Work, North Holland Publishing Company, Amsterdam, New York, Oxford.

Cheifetz, P.N., Gaffud, N. and Dingman, J.F. (1968) Effect of Bilateral Adrenalectomy and Continuous Light on the Circadian Rhythm of Corticotropin in Female Rats. Endocrinology 82: 1117-1124

Chowers, I., Feldman, S. and Davidson, J.M. (1963) Effects of Intrahypothalamic Crytalline Steroids on Acute ACTH Secretion. Amer. J. Physiol. 205: 671-673

Clark, I. (1953) The Effects of cortisone upon Protein Synthesis. J. Biol. Chem. 200: 69-76

Corbin, A., Margili, G., motta, M. and Martin, L. (1965) Effects of hypothalamic and Mesencephalic steroid Implantation on ACTH Feedback Mechanisms. Endocrinology 76: 811-818

Dallman, M.F. and Yates, F.E. (1969) Dynamic Assymmetrics in the Corticosteroid Feedback Path and Distribution Metabolism-binding Elements of the Adrenocortical System. Ann. N.Y. Acad. Sci. 156: 696-721

Daughaday, W.H. and Martz, I.K. (1961) Corticosteroid-binding Globulin: Its Properties and Quantitation. Metabolism 10: 936-956

De Wied, D., Smelik, P.G., Moll, J. and Beuman, P.R. (1964) On the Mechanism of ACTH Release. In "Major Problems in Neuroendocrinology", edited by E. Bajusz and G. Jasmin, pp. 156-176, Karger, Basel.

De Kloet, E.R. and McEwen, B.S. (1976) Glucocorticoid Interacrions with Brain and Pituitary. In "Molecular and Functional Neurobiology", edited by W.H. Gipsen, pp. 257-307, Elsevier, Amsterdam.

Dougherty, T.F., Stevens, W. and Schneebel, G.L. (1973) Functional and Morphological Produced in Target Cells by Anti-inflammatory Steroids. Rec. Progr. Hormone Res. 29: 287-321

Elyakov, G.B., Uvarova, N.I. and Gorshkova, R.P. (1965) The Structure of Carbohydrate Chains of Panaxosides D, E, F. Tetrahedron Letter 51: 4669-4674

Elyakov, G.B., Strigina, L.I., Uvarova, N.I., Vaskovsky, V.B., Dzigenko, A.K. and Kochetkov, N.X. (1964) Glycosides from Ginseng Roots. Tetrahedron Letter 48: 3591-3597

Elyakov, G.B., strigina, L.I., Shapkina , E.V., Aladyina, N.T., Kornilova, S.A. and Dzigenko, A.K. (1968) The Probable Structure of the True Aglycones of Ginseng Glycosides. Tetrahedron 24: 5483-5491

Engel, F.L. (1950) A Consideration of the Roles of the Adrenal Cortex and Stress in the Regulation of Protein Metabolism. Rec. Progr. Hormone Res. 6: 277-313

Feigelson, P., Murphy, L.R. and Colman, P. (1978) Studies on the Cytoplasma Glucocorticoid Receptor and Its Nuclear Interaction in Mediating Induction of Tryptophan Oxygenase Messenger RNA in Liver and Hepatoma. In "Receptor and Hormone Action", edited by B.W. O'Malley and L. Birnbaumer, Vol. 2, Academic Press, New York.

Feldman, S. (1974) Inhibition of Adrenocortical Responses Following Sciatic Nerves Stimulation in Rats with Complete and Partial hypothalamic Deafferentation. In "Progress in Brain Research", edited by W.H. Gipsen, B. Bohus and D. De Wied, Vol. 42, P. 320, Elsevier Scientific Publishing Co., Amsterdam.

Fujita, M., Itokawa, H. and Shibata, S. (1962) Chemical Studies on Ginseng I & II. J. Pharm. Soc. Japan 82: 1634-1641

Ganong, W.F. (1977) Review of Medical Physiology, 8th Edition, Large Medical Publications, Los Altos, California.

Ganong, W.E. and Hume, D.M. (1954) Absence of Stress-induced and Compensatory Adrenal Hypertrophy in Dogs with Hypothalamic Lesions. Endocrinology 55: 474-483

Gommori, R., Miyamoto, F., Shibata, Y., Hihashi, T., Sanada, S. and Shoji, J. (1976) Effect of Ginseng Saponins on Cholesterol Metabolism. II. Effect of Ginsenosides on Cholesterol Synthesis by Liver Slice. Chem. Pharm. Bull. 24: 2985-2987

Gould, N.R. and Siegel, H.S. (1978) Effect of Age and Sex on the Association Constant and Binding Capacity of Chicken Serum for Corticosteroid. Poultry Sci. 57(3): 778-784

Greendyke, R.M., Bradley, E.M. and Swisher, S.N. (1965) Studies on the Effects of Administration of ACTH and Adrenal Corticoeroids on Erythrophagecytosis. J. Clin. Invest. 44: 746-753

Han, B.H., Kim, C.H. and Han, Y.N. (1973) Stimulating Effect of Panax Saponins on the ^{14}C-Leucine Incorporation. Korean Biochem. J. 6(2): 63-71

Hiai, S., Yokoyama, H. and Oura, H. (1979) Stimulation of Pituitary-adrenocortical System by Ginseng Saponin. Proc. Symp. WAKAN-YAKU (Japan) 12: 33-37

Hiai, S., Oura, H., Tsukada, K. and Hirai, Y. (1971) Stimulating Effect of Panax Ginseng Extract on RNA Polymerase Activity in Rat Liver Nuclei. Chem. Pharm. Bull. 19(8): 1656-1663

Hofert, J.F. and White, A. (1968) Effect of A Single Injection of Cortisol on the Incorporation of ^{3}H-Thymidine and ^{3}H-Deoxycytidine into Lymphatic Tissus DNA of Adrenalectomized Rats. Endocrinology 82: 767-776

Hou, J.P. (1977) The Chemical Constituents of Ginseng Plants. Comp. Med. East & West 5(2): 123-145

Hu, S.-Y. (1977) knowledge of Ginseng From Chinese Records. Journal of the Chinese University of Hong kong, Vol. 4, No. 2 : 281-305

Ingle, D.J., Li, C.H. and Evans, H.M. (1946) The Effect of Adrenocorticotropic Hormone on the Urinary Excretion of Sodium, Chloride, Potassium, Nitrogen and Glucose in Normal Rats. Endocrinology 39: 32-42

Jones, M.T., Brush, F.R. and Neama, R.L.B. (1972) Characteristics of Fast Feedback Control of Corticotropin Release by Corticoids. J. Endocrinology 55: 489-497

Jones, M.T., Tiptaft, E.M., Brush, F.R., Fergusson, D.A.N. and Neama, R.L.B. (1974) Evidence for Dual Corticosteroid-receptor Mechanisms in the Feedback Control of Adrenocorticotropin Secretion. J. Endocrinol. 60: 223-233

Karzel, K. (1977) Pharmacological Aspects of Ginseng, A Review. In "Korean Ginseng Studies", edited by H.J. Kim, S.H. Nam and Y. Fukura. pp. 336-345, Il Hwa Co., Ltd., Seoul, Korea.

Kawakami, M., Seto, L., Terasawa, E., Yosida, E., Miyamoto, T., Sekiguchi, M. and Hatter, Y. (1968) Influence of Electrical Stimulation and Lesion in Limbic Structure upon Biosynthesis of Adrenocorticoid in the Rabbit. Neuroendocrinology 3: 337-348

Kendall, J.W., Matsuda, K., Duyck, C. and Gress, M.A. (1964) Studies of the Location of the Receptor Site for Negative Feedback Control for ACTH Release. Endocrinology 74: 279-283

Kim, H.J., Nam, S.H. and Fukura, Y. (1977) Korean Ginseng Studies, published by Laboratory, Il Hwa Co., Ltd., Korea.

Kim, C., Kim, C.C., Kim, M.S., H. C.Y. and Rhe, J.S. (1972) Influence of Ginseng on the Stress Mechanism. Llyoydia 33: 43-48

Knizley, M., Jr. (1972) The Hippocampus and Septal Area as Primary Target Sites for Corticosterone. J. Neurochem. 19: 2737-2745

Lewis, R.A., Kuhlman, D., Delbue, C. Koepf, G.V. and Thorn, G.W. (1940) The Effect of Adrenal Cortex on Carbohydrate Metabolism. Endocrinology 27: 971-982

Li, C.P. and Li, R.C. (1973) An Introductory Note to Ginseng. Amer. J. Chin. Med. 1(2): 249-261

Litwack, G. and Rosenfield, S.A. (1973) Coenzyme Association. A Possible Determination of Short Half-life of Inducible Enzymes in Mammalian Liver. Biochem. Biophys. Res. Comm. 52: 181

Litwack, G. and Rosenfield, S.A. (1975) Liver Cytosol Corticosteroid Binder IB, A New Binding Protein. J. Biol. Chem. 250: 6799-6805

Litwack, G. and Singer, S. (1972) Subcellular Actions of Glucocorticoids, In "Biochemical Actions of Hormones", edited by G. Litwack, Vol. 2, pp. 113-163, Academic Press, New York.

Litwack, G., Filler, R., Rosenfield, S.A., Lichtash, N., Wishman, C.A. and Singer, S. (1973) Liver Cytosol Corticostercoid Binder II, A Hormone Receptor. J. Biol. Chem. 248: 7481-7486

Loeb, J.N. and Stennschein, M.J. (1973) Suppression of Thymidine Incorporation into the Gastric Mucosa of Cortisone-treated Rats: Possible Relation to Glucocorticoid-induced Gastric Ulceration. Endocrinology 92: 1322-1377

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265-275

Mangili, G., Motta, M. and Martini, L. (1966) Control of Adrenocorticotrophic Hormone Secretion, In "Neuroendocrinology", edited by L. Martin and W.F. Ganong, Vol. 1, pp. 297-370, Academic Press, New York.

Martin, C.R. (1976) Glucocorticoids, In "Textbook of Endocrine Physiology", pp. 60-79, The Williams & Williams Company/ Baltimore.

McCann, S.M. and Porter, J.C. (1969) Hypothalamic Pituitary Stimulating and Inhibiting Hormones. Physiol. Rev. 49: 240-284

McEwen, B.S., Magnus, C. and Wallach, G. (1972a) Soluble Corticosterone-binding Macromolecules Extracted from Rat Brain. Endocrinology 90: 217-226

McEwen, B.S., Weiss, J.M. and Schwartz, L.S. (1969) Uptake of Corticosterone by Rat Brain and Its Concentration by Certain Limbic Structures. Brain Res. 16: 227-241

McEwen, B.S., Zigmond, R.E. and Gerlach, J.L. (1972b) Sites of Steroid Binding and Action in the Brain. In "Structure and Function of Nervous Tissue", edited by G.H. Bourne, Vol. 5, pp. 205-291, Academic Press, New York.

Murphy, B.E.P. (1971) Hormone Assay Using Binding Proteins in Blood. In "Principles of Competitive Protein Binding Assays", edited by W.D. Odell & W.H. Daughaday, pp. 108-133, J.B. Lippincott Company, Philadelphia & Toronto.

Nabata, H., Saito, H. and Takagi, K. (1973) Pharmacological Studies of Neutral Saponins (GNS) of Panax Ginseng Root. Japan J. Pharmacol. 23: 29-41

Navarette, J.V., Petit, D.W. (1962) Induced eosinopenia and Basophilopenia by "Free" Steroids in vitro. Acta Endocrinol. 39: 135

Nelson, D.H., Sandberg, A.A., Palmer, J.G. and Tyler, F.H. (1952) Blood Levels of 17-Hydroxycorticoids following the Administration of Adrenal Steroids and their Relation to Circulating Leukocytes. J. Clin. Invest. 31: 843-849

Oura, H., Hiai, S. and Sero, H. (1971) Synthesis and Characterization of Nuclear RNA Induced by Radix Ginseng E xtract in Rat Liver. Chem. Pharm. Bull. 19(8): 1598-1605

Oura, H., Tsukada, K. and Nakagawa (1972a) Effect of Radix Ginseng Extract on Cytoplasmic Polysome in Rat Liver. Chem. Pharm. Bull. 20(2) 219-225

Oura, H., Nakashima, S., Tsukada, K. and Ohta, Y. (1972b) Effect of Radix Ginseng Extract on Serum Protein Synthesis. Chem. Pharm. Bull. 20(5): 980-986

Oura, H., Hiai, S., Odaka, Y. and Yokozawa, T. (1975) Studies on the Biochemical Action of Ginseng Saponin. I. Purification from Ginseng Extract of the Active Component Stimulating Serum Protein Biosynthesis. J. Biochem. 77: 1057-1065

Pegg, P.J. and Keane, P.M. (1969) The Simulataneous Estimation of Plasma Cortisol and Transcortin Binding Characteristics by a Competitive Protein Binding Technique. Steroids 14(6): 705-715

Saffran, M., Schally, A.V. and Benfrey, B.G. (1955) Stimulation of the Release of Corticotropin from the Adrenohypophysis by a Neurohypophysial Factor. Endocrinology 57: 439-444

Saito, H., Morita, M. and Takagi, K. (1973) Pharmacological Studies of Panax Ginseng Leaves. Japan J. Pharmacol. 23: 43-56

Saito, H., Yoshida, Y. and Takagi, K. (1974) Effect of Panax Ginseng Root on Exhaustive Exercise in Mice. Japan J. Pharmacol. 24: 119-127

Sakakibara, K., Shibata, Y., Higashi, T., Sanada, S. and Shoji, J. (1975) Effect of Ginseng Saponins on Cholesterol Metabolism. I. The Level and the Synthesis of Serum and Liver Cholesterol in Rats Treated with Ginsenosides. Chem. Pharm. Bull. (Tokyo) 3: 1009-1016

Sanada, S. and Shoji, J. (1978) Studies on the Saponins of Ginseng. III. Structures of Ginsenoside R_{b3} and 20-Glucoginsenoside-R_f. Chem. Pharm. Bull. 26:1694-1697

Sanada, S., Kondo, N., Shoji, J., Tanada, O. and Shibata, S. (1974a) Studies on the Saponins of Ginseng. I. Structures of Ginsenosides R_0, R_{b1}, R_{b2}, R_c and R_d. Chem. Pharm. Bull. 22: 421-428

Sanada, S., Kondo, N., Shoji, J., Tanaka, O. and Shibata, S. (1974b) Studies on the Saponins of Ginseng. II. Structures of Ginsenosides R_e, R_f and R_{g2}. Chem. Pharm. Bull. 22: 2407-2412

Sayers, G. and Sayers, M.A. (1947) Regulation of Pituitary Adrenocorticotropic Activity During the Response of the Rat due to Acute Stress. Endocrinology 40: 265-273

Scatchard, G. (1949) The Attraction of Proteins for Small Molecules and Ions. Ann. N.Y. Acad. Sci. 51: 660-672

Schapiro, S., Percin, C.J. and Koticha, F.J. (1971) Half-life of Plasma Corticosterone During Development. Endocrinology 89: 284-287

Schimke, R.T. and Doyle, D. (1970) Control of Enzyme Levels in Animal Tissues. Ann. Rev. Biochem. 39: 929-976

Schally, A.V., Anderson, R.N., Lipscomb, H.S., Long, J.M. and Guillemen, R. (1960) Evidence for the Existence of Two Corticotropin-releasing Factors, and . Nature 188: 1192-1193

Seckel, H.P.G. (1940) The Influence of Various Physiological Substances on the Glycogenolysis of Surviving Rat Liver. Endocrinology 26: 97-101

Shibata, S., Fujita, M. and Itokawa, H. (1962) The Stereochemistry of Panaxadiol. Tetrahedron Letters 26: 1239-1242

Shibata, S., Fujita, M., Itokawa, H., Tanaka, O. and Ishii, T. (1963a) Panaxadiol- A Sapogenin of Ginseng Roots (1). Chem. Pharm. Bull. 11: 759-761

Shibata, S., Tanaka, O., Nagai, M. and Ishii, T. (1963b) Studies on the Constituents of Japanese and Chinese Crude Drugs, XII. Panaxadiol, A sapogenin of Ginseng Roots (2). Chem. Pharm. Bull. 11: 762-765

Shibata, S., Tanaka, O., Sado, M. and Tsushima, S. (1963c) On Genuine Sapogenin of Ginseng. Tetrahedron Letter 12: 795-800

Shibata, S., Tanaka, O., Soma, K., Iita, Y., Ando, T. and Nakamura, H. (1965) Studies on Saponins and Sapogenins of Ginseng, The Structure of Panaxadiol. Tetrahedron Letter 3: 207-213

Silber, R.H. and Porter, C.C. (1953) Nitrogen Balance, Liver Protein Repletion and Body Composition of Cortisone-treated Rats. Endocrinology 52: 518-525

Smelik, P.G. and Papaikonomou, E. (1973) Steroid-feedback Mechanism in Pituitary-adrenal Function. In "Drug Effects on Neuroendocrine Regulation, Progress in Brain Research", edited by E. Zimmermann, W.H. Gipsen, B.H. Marks and D. De Wied, Vol. 39, pp. 99-110, Elsevier, Amsterdam.

Stevens, W., Colessides, C. and Dougherty, T.F. (1965) Effects of Cortisol on the Incorporation of Thymidine-2-^{14}C into Nucleic Acids of Lymphatic Tissue from Adrenal-ctomized CBA-mice. Endocrinology 76: 1100-1108

Stevens, W., Reed, D.J., Erickson, S. and Grosser, B.I. (1973) The Binding of Corticosterone to Brain Proteins: Diurnal Variation. Endocrinology 93: 1152-1156

Sung, C.Y. and Chi, H.C. (1958) The Effect of Panax Ginseng on the Adrenocortical Activity in Rats Subjected to Temperature Stress. Acta Physiol. Sinica 22: 155-158, through: Li, C.P. and Li, R.C. (1973) An Introductory Note to Ginseng. Amer. J. Chin. Med. 1: 249-261

Takagi, K. (1977) Pharmacological Studies of Ginseng. In "Korean Ginseng Studies", edited by Kim, H.J., Nam, S.H. and Y. Fukura, pp. 346-357, published by Laboratory, Il Hwa Co., Ltd., Korea.

Takagi, K., Saito, H. and Tsuchiya, M. (1972a) Pharmacological Studies of Panax Ginseng Root. Pharmacological Properties of A Crude Saponin Fraction. Japan J. Pharmacol. 22: 339-346

Takagi, K., Saito, H. and nabata, H. (1972b) Pharmacological Studies of Panax Ginseng Root. Estimation of Pharmacological Actions of Panax Ginseng Root. Japan J. Pharmacol. 22: 245-259

Tanaka, B., Nagai, M. and Shibata, S. (1964) The Stereochemistry of Protopanaxadiol, A Genuine Sapogenin of Ginseng. Tetrahedron Letter 33: 2291-2297

Thompson, E.B. amd Lippman, M.E. (1974) Mechanism of Action Glucocorticoids. Metabolism 23(2): 159-202

Westphal, U. (1971) Steroid-protein Interactions. In "Monographs on Endocrinology", edited by F. Gross, A. Labhart, T. Mann, L.T. Samuels and J. Zander, Vol. 4, pp. 164-293, Springer-Verlag Berlin.

Yahara, S., Kaji, K. and Tanaka, O. (1979) Further Study on Dammarane-type Saponijs of Roots, Leaves, Flowers-buds and Fruits of Panax Ginseng C.A. Meyer. Chem. Pharm. Bull. 27(1): 88-92

Yamamoto, M. (1973) Metabolism & Disease 10: 184, through: Ikehara et al. (1978) Effect of Ginseng Saponins on Cholesterol Metabolism. III. Effect of Ginsenoside-R_{b1} on Cholesterol Synthesis in Rats Fed on High-fat Diet. Chem. Pharm. Bull. 26: 2844-2849

Yard, A.C. and Kadowitz, P.J. (1972) Studies on the Mechanism of Hydrocortisone Potentiation of Vasoconstritor Responses to Epinephrine in the Anesthetized Animal. Europ. J. Pharmacol. 20: 1-9

Yokozawa, T. and Oura, H. (1976) Effect of Ginseng Extract on Lipid and Sugar Metabolism. II. Nutritional states in Rats. Chem. Pharm. Bull. 24(5): 987-990

Yokozawa, T., Semo, H. and Oura, H. (1975) Effect of Ginseng Extract on Lipid and Sugar Metabolism. I. Metabolic correlation between Liver and Adipose Tissue. Chem. Pharm. Bull. 23(12): 3095-3100

Yokozawa, T., Kanai, K., Takefuji, M. and Oura, H. (1976) Effect of Ginseng Saponin on Liver Glycogen Content. Chem. Pharm. Bull. 24(12): 3202-3204

Yokozawa, T., Kitahra, N., Okuda, S. and Oura, H. (1979) Effect of Ginseng Principle on Pyruvate Kinase Activity in Rat Liver. Chem. Pharm. Bull. 27(2): 419-423

Zenker, N. and Bernstein, D.E. (1958) The Estimation of Small Amounts of Corticosterone in Rat Plasma. J. Biol. Chem. 231: 695-701

Zimmermann, E. and Critchlow, V. (1969) Suppression of Pituitary-adrenal with Physiological Plasma Levels of Corticosterone. Neuroendocrinology 5: 183-192